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# **The Vectors and Transmission Routes of Animal Trypanosomiasis on the Jos Plateau north central Nigeria**

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Submitted in fulfilment of the requirements of the degree of Doctor of Philosophy

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# Declaration

I declare that the research described within this thesis is my own work and that this is my own composition and certify that it has never been submitted for any other degree or professional qualification.

Oluwashola Olaniyan

Edinburgh, 2016

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## Abstract

Tsetse flies, *Glossina* species, are the biological vectors of *Trypanosoma* species which cause animal African trypanosomiasis (AAT) in livestock (especially cattle) in sub-Saharan Africa. This disease is often fatal without treatment and negatively impacts on rural, agricultural and economic development.

On the Jos Plateau, north central Nigeria, AAT was historically of little significance due to the presumed absence of tsetse and Fulani pastoralists were encouraged to settle there. But over the last 30 years, the disease has become widespread and highly prevalent in the area. This has been attributed to the expansion of tsetse on the plateau, frequent migrations of cattle to areas with higher tsetse densities and the presence of other biting flies which serve as mechanical vectors.

In the current study, the presence and abundance of tsetse was determined in selected villages using biconical tsetse trap surveys. The low number of flies trapped suggests that tsetse expansion has been very limited within the plateau but the fact that trypanosome DNA was present in over half of these flies implicates them in AAT transmission.

The migration of a herd of cattle was also tracked and during the period, blood samples were collected from the cattle and examined for trypanosomes using molecular techniques. Despite prophylactic treatment and deltamethrin sprays, results showed that a significant proportion of the animals (52%) had become

infected with *T. vivax* over the migration period. Tsetse flies (*G. palpalis*) were also slightly more abundant in some of parts of the migration area.

Potential mechanical vectors (*Stomoxys* spp. and Tabanidae) were trapped and results obtained from the examination of their mouthparts for trypanosomes indicate their involvement in transmission. However, it is difficult to make any definite conclusions about their overall contribution which is thought to be minimal and more studies are needed to clarify their significance.

It is concluded that trypanosomiasis risk from tsetse on the Jos Plateau is currently low and seasonal migration appears to be the main driver of AAT transmission by exposing cattle to more tsetse for longer periods. Other biting flies may play a limited role which remains undetermined. Continued monitoring of cattle and tsetse across the plateau over the next few years is important and the careful use of trypanocides and insecticide treated cattle is recommended as an appropriate control strategy.

## List of Abbreviations

AAT	Animal African Trypanosomiasis
BICOT	Biological Control Project for Tsetse flies
bp	Base pairs
CIDLID	Combating Infectious Diseases in Livestock for International Development
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide-triphosphate
FAO	Food and Agricultural Organization of the United Nations
FTD	Flies per trap per day
GIS	Geographic Information System
GPS	Global Positioning System
HAT	Human African Trypanosomiasis
IAEA	International Atomic Energy Agency
ITS	Internal Transcribed Spacer
LAMP	Loop Mediated Isothermal Amplification
LGA	Local Government Area
NITR	Nigerian Institute for Trypanosomiasis Research
NPC	Nigeria National Population Commission
PCR	Polymerase Chain Reaction
RIM	Resource Inventory and Management

rRNA	ribosomal Ribonucleic Acid
SAT	Sequential Aerial Technique
SIT	Sterile Insect Technique
SOS	Stamp out Sammore
SSA	Sub-Saharan Africa
WHO	World Health Organization
WWF	World Wildlife Fund
ZIP	Zero Inflated Poisson

# **1 Introduction and Literature Review**



## 1.1 Introduction

Tsetse-transmitted animal African trypanosomosis (AAT) also known as nagana, is a severe, wasting disease of livestock in sub-Saharan Africa (SSA) caused by several species of a protozoan parasite genus *Trypanosoma*. The major trypanosome species responsible for AAT include *Trypanosoma vivax*, *T. congolense* and *T. brucei brucei* (O’Gorman *et al.*, 2009). Animals suffering from trypanosomiasis usually become anaemic, progressively lethargic and less productive, and may eventually die if untreated (Losos & Ikede, 1972). Two other sub-species of *T. brucei* also transmitted by tsetse flies, namely: *T. b. gambiense* and *T. b. rhodesiense* are infective to humans, and cause West African or Gambian and East African or Rhodesian Human African trypanosomosis (HAT) respectively (Welburn *et al.*, 2001). West African HAT is a chronic condition whereas *T. b. rhodesiense* causes a more acute disease resulting in death within a few weeks to six months of infection (Odiit *et al.*, 1997). Both forms of the disease are difficult to treat especially if not quickly detected, without treatment result in death (Simaro *et al.*, 2008).

Tsetse flies, *Glossina* species, are the sole biological vectors of the trypanosomes which cause AAT and HAT and thus, the distribution of these diseases is closely associated to that of its tsetse vectors which covers about 10 million km<sup>2</sup> between Longitude 14°N and 29°S of SSA (Rogers & Randolph, 1986). However, animal trypanosomiasis due to *T. vivax* occurs outside of tsetse infested areas in Africa and in South and Central America where it is mechanically transmitted by other haematophagous flies such as *Stomoxys* species and Tabanidae (Hall & Wall, 2004).

Both AAT and HAT occur mainly in rural communities and constitute a major constraint to rural development in 37 tsetse-infested countries of SSA. According to the Food and Agricultural Organization (FAO), around 50 million animals and 60 million people in Africa are at risk from AAT and HAT respectively and annual cattle mortality due to AAT is about three million (FAO, 2014). In terms of agricultural gross domestic product (GDP), total losses due to AAT extrapolated for the tsetse-infested areas of Africa are valued at US\$ 4.75 billion per year (Budd, 1999; FAO, 2014). The economic burden imposed by tsetse and trypanosomiasis in Africa is worsened by the fact that of the 37 countries infested by tsetse, 32 are among the world's poorest nations and they are further impoverished by the direct and indirect losses due to the disease and its control (Ducheyne *et al.*, 2009; Hursey & Slingenbergh, 1995; Swallow, 2000).

Recognition of the immense negative impacts of tsetse and trypanosomiasis on the health and economy of Africa has necessitated the establishment of both small and large scale tsetse and trypanosomiasis control operations in affected countries (Torr *et al.*, 2005). In July 2000, leaders of member nations of the African Union (AU) established the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC); a joint project aimed at eliminating tsetse and trypanosomiasis from the whole continent using an area-wide approach and a combination of available tsetse control techniques (Kabayo, 2002). While this intention is laudable, the practicalities of its implementation raise considerable doubt about its chances of success; especially because of the vast area involved, the unique biology of tsetse and the huge financial and logistics requirements (Rogers & Randolph, 2002).

Nigeria, lies between latitudes 4°N and 14°N and longitudes 2.3°E and 12.5°E in West Africa and is one of the countries affected by the scourges of tsetse, AAT and HAT. Historically, eleven tsetse species were known to occur in Nigeria, occupying about 80% of the country from its extreme south to latitude 12°N (Glover, 1965). However, only four species: *Glossina morsitans submorsitans*, *G. longipalpis*, *G. palpalis palpalis* and *G. tachinoides* were important in trypanosomiasis transmission. The first two species were largely responsible for the transmission of AAT while the latter were the main vectors of HAT (Glover, 1965; Nash, 1969). Between the mid-1920s and 1960s, human and animal trypanosomiasis were rampant and reached epidemic proportions especially in the northern parts of the country where these four tsetse species were widely distributed (Duggan, 1962; Glover, 1965).

The epidemics were controlled by well organised large scale tsetse control operations initially employing ruthless and discriminative clearing of riverine vegetation which formed the main habitats of tsetse and subsequently by ground and aerial spraying of such habitats with residual organochloride insecticides (Davies, 1964, 1971). Apart from targeting the tsetse vectors, the control activities also included active surveillance and treatment of large numbers of people and cattle with trypanocidal drugs, and in some cases, relocation of villages away from areas with heavy tsetse infestations (Glover, 1965; Ford 1971; Nash, 1948). Through these efforts, a large area in the north-east was cleared of tsetse flies rendering about 55.7% of the country tsetse-free and human sleeping sickness was eliminated from many of the formerly affected areas (Davies, 1964, 1971; Ikede *et al.*, 1987; Onyiah, 1983).

Currently, the problems of tsetse and trypanosomiasis in Nigeria are less severe owing to significant declines in tsetse populations across the country attributed not only to the above-mentioned tsetse control operations, but also to the destruction of natural tsetse habitats and wild hosts which accompanied the rapid expansion of the human population and agricultural activities (Bourn *et al.*, 2001). This has led to the disappearance of *G. m. submorsitans* and *G. longipalpis* from most of the previously occupied areas while *G. tachinoides* and *G. palpalis* only persist at very low densities in fragmented habitats (Bourn *et al.*, 2001; Omoogun *et al.*, 1991). Indeed, during a recent tsetse survey in preparation for area-wide tsetse control under the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), *G. tachinoides* was not found in Jigawa State in the north-east where it was once abundant as most of the riverine vegetation which constituted its habitat had been destroyed (Dede *et al.*, 2013).

The decline in tsetse populations across the country has led to a significant reduction in the prevalence and transmission of HAT which is currently limited to Abraka; a historical focus in the delta of the River Niger in Southern Nigeria. Even in this focus, cases are rare and according to World Health Organization records, only 177 cases were reported in the last 13 years and just 10 of these occurred within the last seven years (WHO, 2014). Conversely, animal trypanosomiasis occurs widely and prevalence also varies widely; ranging from 1.9% - 46% in different parts of the country (Ogunsanmi *et al.*, 2000; Pollock, 2001; Omotainse *et al.*, 2004). The widespread nature of the animal disease is probably due to the existence of residual pockets of tsetse infestation and the extensive grazing of cattle owned by nomadic

and semi-nomadic Fulani pastoralists who migrate through areas where tsetse are still present. Availability and easy access to trypanocidal drugs, allows infected animals to be treated by their owners, and mortality due to AAT is relatively low (Oluwafemi, 2007). The presence of trypano-susceptible zebu cattle on a year-round basis, in southern areas of the country from which they were previously excluded due to the risk posed by tsetse (Blench, 1994), is another indicator of a generally improved situation

But despite these apparent improvements, Omotainse *et al.* (2004) estimated that AAT still costs the Nigerian economy about US\$135 million per annum due to its negative effects on cattle weight gain, growth rates, milk yields and reproduction and by its discouragement of the use of draught animals in arable farming. There are also indications that some of the areas previously cleared of tsetse in the north-east of the country have become re-infested since tsetse control activities in these areas were not sustained (Bature, 1995). Furthermore, the presence of tsetse has also been reported on the previously uninfested Jos Plateau (Dede *et al.*, 2005; Kalu, 1996b; Majekodunmi, 2006) and AAT is now endemic and widespread in this area with attendant negative effects on animal productivity and pastoral livelihoods (Dede *et al.*, 2005; Majekodunmi *et al.*, 2013; Majekodunmi *et al.*, 2014).

This thesis is an investigation of the vectors and transmission of AAT in this recently infested area located within the northern guinea savannah vegetation zone of north central Nigeria. The Jos Plateau was presumably free of tsetse flies due to its high

average altitude (~1280m above sea level) and generally low temperatures and as such, trypanosomiasis was also largely absent from the area (Pullan, 1980). These factors, as well as the availability of abundant grasslands suitable for extensive grazing of livestock, drew large numbers of Fulani pastoralists and their cattle from the surrounding tsetse-infested lowlands onto the plateau starting from the early 20<sup>th</sup> century (Awogbade, 1983; Morrison, 1982 cited in Blench, 1994). Over time, their population has increased considerably and an estimated 300,000 settled Fulani pastoralists who own a total of about 1.07 million cattle (approximately 7% of the national herd) currently live on the Jos Plateau (RIM, 1992) making it an important contributor to the cattle production sector in Nigeria.

From around the early 1980s and onwards, studies began to indicate that animal trypanosomiasis was becoming prevalent on the Jos Plateau (Joshua, 1982; Joshua & Shanthikutmar, 1989; Kalu, 1991) and it was suggested that tsetse were involved in its transmission even though the plateau was at the time, supposedly tsetse-free. Subsequently, tsetse surveys conducted in different parts of the plateau (Dede *et al.*, 2005; Kalu, 1996a, 1996b; Majekodunmi, 2006) confirmed the presence of *G. palpalis* and *G. tachinoides* which were believed to have invaded the plateau about the same time that AAT was first reported. However, these presumptions were incorrect as shown by observations made during outbreaks of HAT on the plateau in 1927 and 1944, which indicate that, at least *G. palpalis* was present on the plateau much earlier (Taylor, 1930; Nash, 1948). This point is discussed further in a later section which reviews in more detail, the history of tsetse and trypanosomiasis on the Jos Plateau.

Regardless of when tsetse invaded the plateau, animal trypanosomiasis is now endemic and widespread in livestock (specifically cattle) on the Jos Plateau and is regarded by pastoralists as a major cause of reduced productivity and occasional mortality among their animals (Kalu, 1996b; Dede *et al.*, 2005; Majekodunmi *et al.*, 2013). In a recent study, Majekodunmi *et al.* (2013) reported an overall AAT prevalence of 46.8% (95% CI: 39.0 – 54.5%) in a sample of 7142 cattle belonging to Fulani pastoralists spread across 30 villages on the plateau. This represents the highest prevalence of AAT ever recorded in this area and raises serious concerns about the impact of AAT on livestock productivity and the livelihoods of pastoralists. It also provides justification for further investigations of the reasons behind this rise in prevalence and the development of appropriate measures to limit the spread of the disease.

Therefore in 2010, a project tagged Stamp out Sammore<sup>1</sup> (SOS) was conceived, with the overall objective of developing and implementing an integrated control programme which would lead to a reduction of the AAT burden on the plateau. However, to develop an effective control strategy, adequate understanding of the factors responsible for the current state of the disease is needed. Of particular relevance would be the description of the current distribution of the vectors, and the various transmission routes and cycles of the disease in the area.

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<sup>1</sup> Sammore means trypanosomiasis in Hausa, an indigenous language widely spoken in northern Nigeria.

The current idea as to why AAT is now prevalent and widespread on the plateau centres on three factors directly related to disease transmission. These are: the so-called recent invasion of the plateau by tsetse flies and a possible expansion in their distribution, the seasonal migration of cattle from the plateau to areas which are likely to be infested by tsetse and mechanical transmission by other biting flies which are common on the plateau (Dede *et al* 2005; Kalu, 1991; Majekodunmi *et al.*, 2013). However, these ideas are mostly speculative and there is little direct evidence to support them. For example, no studies have been carried out to determine whether or not there has been an expansion of tsetse on the plateau and previous tsetse surveys have often focused on single villages in which outbreaks of AAT occurred (e.g. Kalu, 1991; 1996a) or a few villages. Besides, there are no maps of previous tsetse distribution on the plateau against which comparisons can be made. Other biting flies are present, but their involvement in mechanical transmission remains unconfirmed and the seasonal migration of Fulani cattle as a risk factor for AAT has also not been adequately investigated.

Therefore, the work presented in this thesis is intended to provide a better understanding of tsetse distribution and the transmission of animal trypanosomiasis on the plateau. It investigates tsetse distribution and abundance in selected villages using trap surveys and examines the possibility of mechanical transmission also by trapping other biting flies and screening them for the presence of trypanosomes using molecular techniques. It also investigates the role played by seasonal migration of cattle by determining whether tsetse flies are present in areas where cattle migrate to, and if migrating cattle become infected with trypanosomes which are then imported



onto the plateau. The prevalence and diversity of trypanosomes in tsetse trapped on the plateau and in migration areas including a nearby game reserve, is also determined as an indication of disease risk. The prevalence of tsetse endosymbionts which are known to affect tsetse's susceptibility to trypanosomes is also investigated. Based on the results obtained from these studies, some conclusions are drawn about the transmission and spread of AAT on the plateau and recommendations made for possible strategies to limit its continued spread in order to protect pastoral livelihoods.

## **1.2 Literature Review**

The first part of this review provides an overview of the epidemiology of animal trypanosomiasis with respect to the causative organisms and their pathogenic effects on infected hosts, especially cattle. Next, it discusses the tsetse fly vectors, highlighting some of the factors that affect their ability to transmit trypanosomes. Mechanical transmission by other biting flies is also reviewed. Subsequent sections focus on the Jos Plateau with respect to the emergence and spread of animal trypanosomiasis and provide additional background to the investigations carried out in the succeeding chapters of this thesis. It concludes by setting out the aims and objectives of the study as well as providing an outline of the thesis.

### **1.2.1 African Animal Trypanosomiasis**

AAT also known as nagana is a disease of livestock (especially cattle) with wide occurrence in SSA. It is present in all 37 sub-Saharan African countries infested by tsetse flies and has been described as a major impediment to rural and economic

development on the African continent. It is caused by several species of a protozoan parasite belonging to the genus *Trypanosoma* transmitted by *Glossina* species (commonly known as tsetse flies).

#### **1.2.1.1 The African trypanosomes- causative organisms of AAT**

Tsetse-transmitted African trypanosomes belong to the sub-kingdom Protozoa, class Zoomastigophora, order Kinetoplastida, family Trypanosomatidae, section Salivaria and genus *Trypanosoma*. They are classified primarily on the basis of morphological features. Hoare (1970) grouped the known salivarian trypanosomes into subgenera and subspecies based on the sites of their cyclical development in the tsetse vector and provided information on the vertebrate hosts in which they occur. Recent biochemical and molecular characterization has led to the identification of three distinct sub-types of *T. congolense* i.e. Savannah, Riverine/Forest and Kilifi or Kenya coast (Gashumba *et al.*, 1988); one sub-type of *T. simiae* (Tsavo) (Majiwa *et al.*, 1993; Gibson *et al.*, 2001), as well as another species *T. godfreyi*; first recorded in *G. m. morsitans* in Gambia (McNamara *et al.*, 1994). Table 1.1 shows the classification of the tsetse-transmitted African trypanosomes.

**Table 1.1: Classification of African Trypanosomes based on morphology and sites of cyclical development in tsetse after (Hoare, 1970) and recent molecular characterization**

Subgenus	Species/subspecies	Site of development in tsetse	Host Range
<i>Duttonella</i>	<i>T. vivax</i>	Proboscis only	Wild and domestic animals
<i>Nannomonas</i>	<i>T. congolense</i> savannah <i>T. congolense</i> Kilifi <i>T. congolense</i> Forest  <i>T. simiae</i> <i>T. godfreyi</i>	Mid-gut and Proboscis	Wild and domestic animals  Wild and domestic Suids Wild and domestic Suids
<i>Trypanozoon</i>	<i>T. brucei brucei</i> <i>T. b. rhodesiense</i> <i>T. b. gambiense</i>	Mid-gut and salivary glands	Man, wild and domestic mammals
<i>Pycnomonas</i>	<i>T. suis</i>	Mid-gut, salivary glands and proboscis	Suids

*T. evansi* and *T. equiperdum* are also Salivarian trypanosomes of the subgenus *Trypanozoon* but were not included in the above table as they are not transmitted by tsetse. *T. evansi* is mechanically transmitted by *Stomoxys* and Tabanidae, and causes a disease known as surra in a wide variety of domestic animals including camels, cattle, small ruminants, dogs, pigs and water buffalo, (Franke *et al.*, 1994; Verloo *et al.*, 2000 in Luckins & Dwinger, 2004). *T. equiperdum* is sexually transmitted in equines (horses and donkeys) and causes a usually chronic but fatal condition known as dourine which is endemic in Africa, North Africa, the Middle East, South

America, Eastern Europe and Indonesia (Stevens and Brisse, 2004). However, of all the tsetse-transmitted animal infective trypanosomes, the species most commonly responsible for AAT in cattle are *Trypanosoma vivax*, *T. congolense* and *T. brucei* *brucei* (O' Gorman *et al.*, 2009).

#### **1.2.1.2 Pathogenic effects and clinical signs of AAT**

As there are many species and sub-species of trypanosomes which are infective to a wide range of animal hosts, there is considerable variability in the pathogenic effects and course of the disease (s) they cause in their hosts (Losos & Ikede, 1972). Only the effects and clinical course of those species commonly found in cattle- *T. vivax*, *T. congolense* and *T. brucei* s. l. are considered in this section.

An infection is initiated when infective trypanosomes are injected into the animal host by a tsetse fly during a blood meal. The pre-patent period (time between inoculation of metacyclic trypanosomes and the detection of parasites in the peripheral circulation) ranges from 1-3 weeks depending on the virulence of the infecting trypanosome, the infective dose, and the immune status of the host (reviewed by Taylor & Authié, 2004). Typically, experimental infections progress in three successive stages: acute, stabilization and chronic, but natural infections observed in the field may present many different and more complex scenarios (Losos & Ikede, 1972).

The early acute phase is characterized by the continuous presence of trypanosomes in the blood at detectable concentrations ( $10^3 - 10^8/\text{ml}$ ) and is often accompanied by fever which is highest during the first peak of parasitaemia and fluctuates thereafter with waves of parasitaemia (Murray *et al.*, 1981). Anaemia, which is the most prominent feature of animal trypanosomiasis, develops with the onset of parasitaemia and may be observed as pallor of the mucous membranes and by a marked reduction in the packed cell volume (PCV) which may fall from a normal value of 30% or above to 15% after 2-3 months of infection with *T. congolense* or *T. vivax* (Losos & Ikede, 1972; Murray *et al.*, 1981). The severity of anaemia is often affected by the virulence of the infecting parasite population and the age, nutritional status and breed of the host and is correlated with the loss of productivity in infected livestock (Losos & Ikede, 1972; Murray *et al.*, 1981).

Weight loss, weakness, lethargy, loss of condition, reduced milk production and abortion are commonly observed and calves born to infected cattle are often small and weak, resulting in high rates of neonatal mortality. In the terminal stages of the acute disease, animals become extremely weak and recumbent and death may occur within a few weeks to several months (Losos & Ikede, 1972; MacLennan & Na'isa, 1970). Under conditions of good nutrition, animals with infected by less virulent strains may show some degree of resistance and completely recover from clinical disease. But in most cases, animals that survive the acute disease enter into a chronic phase (Murray & Trail, 1984). This phase may be characterized by low-grade parasitaemia, anaemia, cachexia, poor productivity and infertility (Sekoni *et al.*, 1990a, 1990b).

Generally, *T. vivax* is considered the most important cattle trypanosome in West Africa where it often causes acute disease. However, many chronic infections occur and West African strains of *T. vivax* have been associated with high mortality rates and a more rapid progression (Stephen, 1970). In East Africa, *T. congolense* is seen as the more important species but in this region, particular stocks of *T. vivax* have also been known to cause a haemorrhagic disease unlike that of typical *T. vivax* infections (Hudson, 1944). This form of the disease is characterised by high parasitaemia, severe anaemia, enlargement of the spleen and extensive haemorrhage into viscera and at external mucosal surfaces (Assoku & Gardiner, 1989). First reported in Kenyan cattle in 1944 (Hudson, 1944), it has since been reported in central, and coastal provinces of Kenya (Gardiner *et al.*, 1989, Welde *et al.*, 1983) and south eastern Uganda (Magona *et al.*, 2008). Indigenous African cattle infected with *T. brucei* s.l. show no obvious clinical signs of disease although exotic breeds and their crosses may exhibit susceptibility to this parasite (Jordan, 1986). Asymptomatic *T. brucei* infections in cattle may involve human infective *T. b. rhodesiense* and provide an important reservoir of East African human sleeping sickness (Welburn *et al.*, 2001b).

#### **1.2.1.3 Diagnosis of AAT**

Diagnosis of AAT is carried out mainly through the detection of trypanosomes in the blood of infected animals using one of several parasitological techniques. These include the examination of wet and stained thin or thick blood films. More sensitive molecular techniques which are capable of detecting infections which might go undetected using parasitological tests have also been developed. These are based on

the amplification of parasite DNA sequences using either specific or generic primers in polymerase chain reaction (PCR) assays. Species-specific PCRs are time consuming and expensive as several tests are required to detect the various trypanosome species whereas generic PCRs can detect as many as 11 different trypanosome species and subspecies in a single reaction (Njiru *et al.*, 2005). Such tests may, however, have less sensitivity compared to species specific PCRs (Ahmed *et al.*, 2013). The PCR-based diagnostic tests for trypanosomiasis can only be carried out in expensive laboratories where there is constant power supply and a cold-chain can be maintained to preserve the heat sensitive PCR reagents. As such, they have been not of much application in the field diagnosis of AAT even though they are now commonly used in epidemiological surveys where blood samples are transported to and processed in laboratories. However, efforts are being made to develop simple molecular techniques that can be used under field conditions. An example is the loop-mediated isothermal amplification (LAMP) technique which has been trialled with some success (Njiru *et al.*, 2008; Thekisoe *et al.*, 2007) although it is yet to be widely employed in epidemiological studies.

#### **1.2.1.4 Chemotherapy of AAT**

Chemotherapy plays a central role in the control of AAT and about 35 million doses of trypanocidal drugs are used in Africa each year (Holmes *et al.*, 2004). Only three drugs: isometamidium chloride (ISM), homidium, and diminazene aceturate are currently used against AAT and they have been in use for over 50 years (Geerts *et al.*, 2001). Isometamidium chloride is used primarily as a prophylactic which can be applied routinely to all animals in a herd at regular intervals or limited to valuable or

vulnerable stock (e.g. draught bulls or milking cows) when the number of animals succumbing to infection reaches a predetermined threshold. The drug can provide up to six months protection against low to moderate tsetse challenge but this period may be shorter (approximately three months) when challenge is higher (Magona *et al.*, 2004). Homidium has limited prophylactic properties but is used mainly as a therapeutic agent while diminazene aceturate is only used for therapeutic purposes.

In the past, only a few large European companies produced these drugs and their use was strictly regulated by government veterinary departments. Presently, however, many generic forms of these trypanocides are widely available, and with the liberalization of veterinary services and supplies in many African countries, they are bought and administered by individual farmers (Geerts & Holmes, 1998). Many of these farmers are poorly educated and have no access to adequate diagnostic facilities, and they have been known to use sub-standard drugs or wrong dosage and treatment regimens (Grace *et al.*, 2009; McDermott *et al.*, 2000). Such practices are believed to have contributed to the emergence of drug resistant trypanosome strains in many African countries (Clausen *et al.*, 1992; Geerts & Holmes, 1998). Careful and correct use of trypanocides is therefore, important in order to ensure that they remain relevant in the control of animal trypanosomiasis in Africa. Alternation of treatments using isometamidium chloride and diminazene aceturate as a sanative pair is one strategy employed to delay the development of drug resistant trypanosome strains (Whiteside, 1962). Minimizing drug use by reducing contact between livestock and tsetse vectors and intensifying vector control activities has also been



suggested as an effective means of preventing or delaying the emergence of trypanocidal drug resistance (Holmes *et al.*, 2004).

### **1.2.2 Tsetse Flies- vectors of African trypanosomes**

Tsetse flies are the vectors of pathogenic African trypanosomes which cause AAT and HAT. Both male and female tsetse feed exclusively on vertebrate blood and can acquire infective trypanosomes which undergo cyclical development within the tsetse fly and are transmitted to other vertebrate hosts during subsequent feeds.

#### **1.2.2.1 Classification of tsetse flies**

Tsetse flies belong to a single genus, *Glossina*, in the family Glossinidae of the insect Order, Diptera. They were first described and assigned to this genus by Weidemann (1830) (cited in Leak, 1999) and remain one of the most studied groups of insects up till the present time (Leak, 1999). There are 31 extant species and subspecies of tsetse flies classified largely on the basis of morphological differences; especially in the structure of the genitalia (Mulligan, 1970 updated by Jordan, 1993). Tsetse flies are further divided into three sub-genera or groups based on morphology and habitat preferences. These are: *Austenina*, *Nemorhina* and *Glossina* or, *fusca* (forest), *palpalis* (riverine) and *morsitans* (savannah) tsetse respectively. Table 1.2 shows all known tsetse species and sub-species according to their respective sub-genera.

**Table 1.2: Tsetse species and subspecies by sub-genera**

Subgenus <i>Nemorrhina</i> ( <i>Palpalis</i> /Riverine group)	Subgenus <i>Austenina</i> ( <i>Fusca</i> /Forest group)	Subgenus <i>Glossina</i> ( <i>Morsitans</i> /Savannah group)
<i>G. caliginea</i> <i>G. fuscipes fuscipes</i> <i>G. fuscipes martini</i> <i>G. fuscipes quanzensis</i> <i>G. pallicera newsteadi</i> <i>G. pallicera pallicera</i> <i>G. palpalis gambiensis</i> <i>G. palpalis palpalis</i> <i>G. tachinoides</i>	<i>G. brevipalpis</i> <i>G. frezili</i> <i>G. fusca congolensis</i> <i>G. fusca fusca</i> <i>G. fuscipleuris</i> <i>G. haningtoni</i> <i>G. longipennis</i> <i>G. medicorum</i> <i>G. nashi</i> <i>G. nigrofusca hopkinsi</i> <i>G. nigrofusca nigrofusca</i> <i>G. schwetzi</i> <i>G. severini</i> <i>G. tabaniformis</i> <i>G. vanhoofi</i>	<i>G. austeni</i> <i>G. longipalpis</i> <i>G. morsitans centralis</i> <i>G. morsitans morsitans</i> <i>G. morsitans submorsitans</i> <i>G. pallidipes</i> <i>G. swynnertoni</i>

Source: (Leak, 1999).

The development and application of molecular genetic techniques to tsetse taxonomy has led to a review of the sub-species grouping of *Glossina austeni* which is now placed in a fourth group, *Machadomyia* as proposed by Chen *et al.* (1999).

#### 1.2.2.2 Tsetse reproductive biology

Tsetse flies differ from most insects in producing offspring by the method of adenotrophic viviparity in which females lay a single, fully developed larva rather than eggs. Female tsetse flies usually mate with males within the first three days of emergence and once mated, remain fertile for the rest of their life span. Sperm are stored in the spermathecae and used to fertilise eggs ovulated one at a time. The fertilised egg is retained within the uterus and hatches into a first stage larva which is nourished by milk glands until it is fully developed. The gravid female then searches

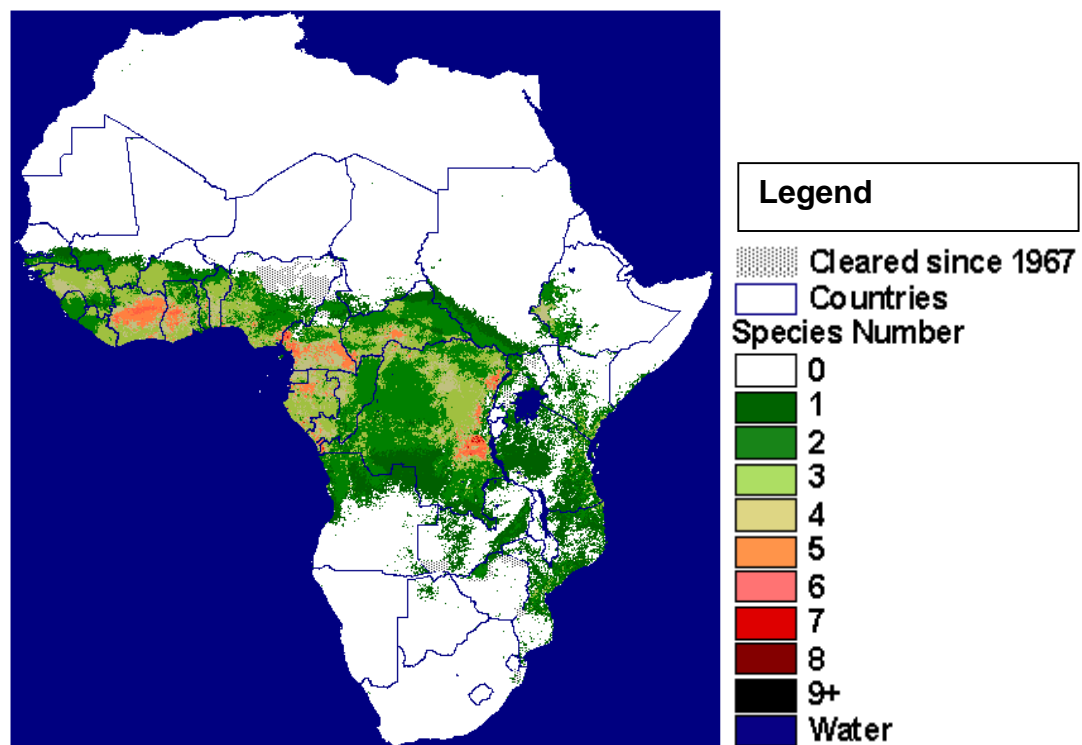
for soft, moist soil where it deposits the fully developed larva which burrows a few metres into the soil and forms a puparial case within a few minutes. The pupal period is temperature dependent and adult emergence occurs about 30 days later at 24°C (Glasgow, 1963). The whole life cycle takes between 30-60 days. On the average, each mature female produces a single larva at intervals of between nine and 10 days (Leak, 1999).

#### **1.2.2.3 Tsetse distribution**

Tsetse flies are distributed discontinuously over approximately 10 million square kilometres of SSA between latitudes 14 °N and 29 °S. The discontinuous nature of their distribution is related to their requirement for particular habitats that range from evergreen forests to dry savannahs (Rogers & Randolph, 1986). The overall limits of their distribution are determined principally by climatic factors such as rainfall, temperature and humidity. These factors are in turn influenced by altitude and vegetation which are able to mitigate the severity of climate (Nash, 1969; Rogers & Randolph, 1993). At the northern edge of their distribution, tsetse flies are limited by high temperature and dryness whereas the southern limits of distribution seem to be determined by seasonal low temperatures (Rogers & Randolph, 1986).

Early descriptions of tsetse distribution were based on presence/absence data derived from trap or transect surveys which in combination with data on vegetation type, meteorological records and altitude were used to make estimates of the distributional limits of many species. These resulted in the production of national and regional maps of tsetse distribution such as those of Ford and Katondo (1977). In recent

times, the application of modern techniques of remote sensing, geographical information systems (GIS) and geo-statistical modelling has enabled predictions of continent-wide tsetse distribution although field surveys may be required to verify the accuracy of these predictions especially at smaller spatial scales (Rogers, 2000; Wint & Rogers 2000). Using these methods, tsetse distribution maps have been produced for the whole of Africa and one of such maps is shown in Figure 1.1.



**Figure 1.1: Predicted areas of tsetse distribution in Africa**

(Source: <http://ergodd.zoo.ox.ac.uk/tseweb/allspecies.htm>, accessed 20<sup>th</sup> October 2014).

#### **1.2.2.3.1 Changing distribution and decline of tsetse across Africa**

According to Rogers and Randolph (1986), there has been little change in the total area occupied by tsetse in Africa despite several decades of control efforts. However,

changes have been observed at smaller scales in several countries such as Nigeria, Gambia, Kenya, Ethiopia and Zimbabwe where tsetse populations have generally declined and some species, especially of the *morsitans* group have been locally eliminated (Bourn *et al.*, 2001). These local changes have been attributed to rapid and sustained growth in human populations with resultant changes in land use leading to the destruction of tsetse habitat and elimination of their wild hosts except in protected areas such as game reserves and national parks (Van den Bossche *et al.*, 2010).

It has been suggested that this non-deliberate or autonomous tsetse control, could lead to the extinction of several species of tsetse in many parts of Africa over the next 20 years (Reid *et al.*, 2000; Van den Bossche *et al.*, 2010). It is, however, noted that species of the *palpalis* group, particularly *G. p. palpalis* and *G. tachinoides* are less affected by these developments and persist in many areas because of their ability to adapt to peri-domestic, fragmented and other man-made habitats (Dede *et al.*, 2005; de la Roque *et al.*, 2001). However, despite their adaptability, studies have shown that habitat fragmentation due to clearance of riverine vegetation to provide space for cultivation and other human activities can lead to reductions in the density, and changes in the distribution of this group of flies (Dede *et al.*, 2013; de La Roque *et al.*, 2001).

#### **1.2.2.3.2 Seasonal variations in tsetse distribution and density**

Tsetse fly populations often become redistributed within their habitats in response to seasonal changes in environmental conditions such as increasing dryness and heat

(Nash & Page, 1953). This is particularly common among the *palpalis* and *morsitans* group flies in drier areas of the northern savannas where significant seasonal climatic changes occur. In such areas, flies concentrate in the shade provided by dense riverine vegetation at the height of the dry season when conditions outside of these areas could be rapidly lethal and disperse along the length of rivers or into less densely wooded surrounding areas during the wet season when conditions become cooler and more humid (Nash & Page, 1953). Van den Bossche & De Deken (2001) reported significant seasonal changes in the distribution and abundance of *G. m. morsitans* in cultivated areas of eastern Zambia. They found that the density of flies increased at the start of the rains and peaked towards the end of this season. The fly became less abundant during the dry cold months in particular. These seasonal changes in tsetse distribution and abundance also correlated with changes in the abundance of cattle which were the main hosts of the fly.

Seasonal changes observed in tsetse distribution often have implications for disease transmission and epidemiology. For example, the use of permanent pools on shaded portions of streams and rivers during the dry season as watering points for cattle, or water collection points by villagers, often brings cattle and humans into close personal contact with tsetse. This leads to increased transmission and many of the sleeping sickness outbreaks that occurred in parts of Northern Nigeria between the mid-1920s and 1960s resulted from such close personal man-tsetse fly contact (Duggan, 1962; McLetchie, 1953).

#### 1.2.2.4 Tsetse Habitats

The three groups of tsetse flies (*morsitans*, *palpalis* and *fusca*) occupy different types of habitat. The *fusca* or forest tsetse flies are usually confined to rainforests in West, Central and East Africa but may also occur in isolated forest patches or riverine forests in the savannah zones (Nash, 1969). An exception within this group is *G. longipennis* which occurs in the dry rangelands of East Africa. The members of the *fusca* group usually have little contact with humans or livestock in the dense forest habitats where they live and are not considered to be of significant importance as vectors of animal or human trypanosomiasis (Nash, 1969). However, as humans and cattle encroach on forest habitats, tsetse in this group could become more important in disease transmission as was shown for *G. tabaniformis* and AAT transmission in Gabon and Zaire (Leak *et al.*, 1991).

The habitats of riverine or *palpalis* group tsetse are mainly forests and different kinds of riverine vegetation which provide adequate shade. Their distribution often extends along the vegetation on rivers, streams and lake shores into semi-arid woodland savannas (Ford, 1971). Two species of this group, *G. tachinoides* and *G. p. palpalis* have also been found living and breeding in atypical peri-domestic and other man-made habitats such as orchards and plantations as long as these provide suitable shade and a ready supply of hosts. Peri-domestic behaviour has been reported in *G. palpalis* in northern Nigeria (Ahmed, 2004), *G. tachinoides* in southern Nigeria (Baldry, 1964) and *G. tachinoides* on the Jos Plateau in north-central Nigeria (Dede *et al.*, 2005). As a result of their association with habitats regularly used by man and livestock, three of the more widespread species in this group (*G. palpalis*, *G. fuscipes*

and *G. tachinoides*) are very important vectors of HAT and AAT (Bourn *et al.*, 2001).

Tsetse flies of the *morsitans* or savanna group are the most widely distributed in Africa; typically occurring in wooded grasslands, they can also be found in forest edges, scattered thickets or even open country (Nash, 1969; Leak, 1999). Due to their widespread distribution, they are considered the most important vectors of AAT in Africa (Bourn *et al.*, 2001). Two species of this group (*G. m. submorsitans* and *G. longipalpis*) occur in Nigeria but their distribution is currently very restricted; limited to protected areas such as the Kainji Lake National Park (KLNP) in Kwara and Niger States and Yankari Game Reserve in Bauchi State where wildlife hosts are abundant and human habitation and activities such as farming, wood felling and hunting are excluded (Ajibade and Agbede, 2011).

#### **1.2.2.5 Tsetse hosts and feeding patterns**

Both sexes of tsetse feed only on vertebrate blood and require a blood meal on average, once every 3-4 days (Hargrove & Williams, 1995; Leak, 1999; Randolph, *et al.*, 1991). Knowledge of tsetse fly hosts and feeding patterns is important in understanding the epidemiology of tsetse-transmitted trypanosomiasis and aids the selection of appropriate vector and disease control strategies (Van den Bossche & Staak, 1997). However, observing the feeding habits of tsetse flies in nature is difficult and as such, identifying the source of residual blood meals in wild tsetse flies has provided an indirect means of obtaining information on the hosts and feeding patterns of tsetse (Weitz, 1963). Various serological techniques (Tarimo *et*



*al.*, 1985; Weitz, 1963) and more recently, molecular assays based on the polymerase chain reaction (PCR) (e.g. Muturi *et al.*, 2011; Steuber *et al.*, 2005) have been used to identify tsetse blood meal sources.

The results of these studies demonstrate that tsetse flies feed on a wide variety of vertebrate hosts which includes reptiles, birds and many mammalian species and that some species/groups of tsetse feed preferentially on certain animal species or groups. Weitz (1963) evaluated the results of several studies on tsetse hosts and feeding patterns conducted between 1952 and 1962 involving the identification of 22,000 blood meals from 15 *Glossina* species and grouped the flies into five feeding categories as shown in Table 1.3.

**Table 1.3: Tsetse feeding categories according to (Weitz, 1963)**

Feeding Category	Tsetse species
Tsetse feeding mainly on Suidae	<i>G. swynnertoni</i> , <i>G. austeni</i> , <i>G. tabaniformis</i> and <i>G. fuscipleuris</i>
Tsetse feeding equally on Suidae and Bovidae	<i>G. morsitans morsitans</i> , <i>G. m. submorsitans</i>
Tsetse feeding mainly on Bovidae	<i>G. pallidipes</i> , <i>G. longipalpis</i> and <i>G. fusca</i>
Tsetse feeding mainly on mammals other than pigs or Bovids	<i>G. longipennis</i> and <i>G. brevipalpis</i>
<i>Glossina</i> feeding on most available hosts and man	<i>G. palpalis</i> and <i>G. tachinoides</i>

Other studies have however, revealed that tsetse feeding patterns are not always fixed and depend on a variety of factors such as the availability and abundance of hosts and the habitat in which both tsetse and hosts live. For example, Van den Bossche

and Staak (1997) found that *G. m. morsitans*, which according to Weitz (1963) and earlier studies in Zambia (Baldry, 1987; Okiwelu, 1977) fed predominantly on Suidae, took 75% of its blood meals from cattle in an area of Zambia where cattle were more abundant than wild game and other domestic animals. In another study conducted during a tsetse control program in Zimbabwe, the favored hosts of *G. m. morsitans* such as warthog, bushpig, kudu and bushbuck were selectively eliminated and the fly switched to other available hosts especially Bovids and elephants (Cockbill, 1972 in Jordan, 1986).

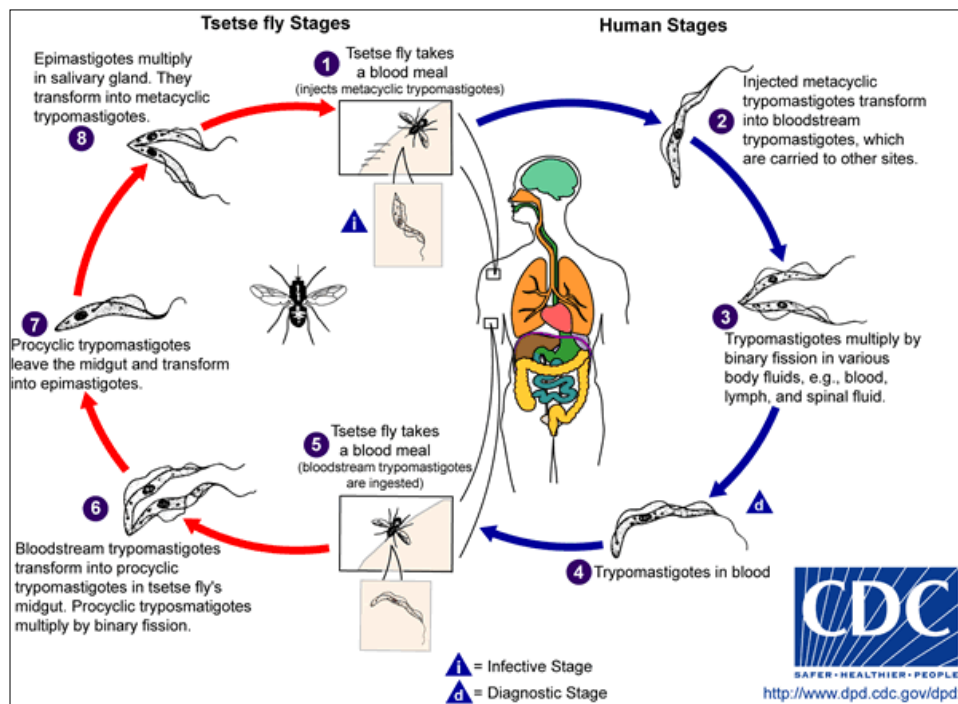
Tsetse hosts and feeding preferences can also vary between regions and may or may not depend on host availability in an area. As an example of this, Jordan *et al.* (1962) found that majority of *G. tachinoides* fed on man in northern Nigeria were taken from man whereas, Baldry (1964) found that in southern Nigeria, this tsetse species preferred pigs over man even when human hosts was apparently more available. It is also notable that some tsetse species, especially in the *palpalis* group, are more flexible and opportunistic in their feeding habits and utilize the most abundant or most commonly encountered hosts in their habitats (Weitz, 1963). The wide range of host choice and plasticity in feeding patterns exhibited by this group of flies is perhaps part of the reason for their persistence in the face of massive environmental changes which have eliminated much of their natural habitats in many parts of Africa and should be taken into consideration when planning tsetse and trypanosomiasis control programmes.

#### **1.2.2.6 Tsetse and the transmission of trypanosomes**

Tsetse flies have been associated with African trypanosomiases since the discovery by Sir Robert Bruce over a century ago, that they were the vectors of the trypanosomes which caused the animal disease which the Zulu people called “nagana” (Steverding, 2008). Since then, considerable progress has been made in uncovering the complex interactions between tsetse flies and trypanosomes as well as the various factors influencing these relations (reviewed by Aksoy *et al.*, 2003). More research is currently being conducted to fully elucidate the interactions which occur during the cyclical development of trypanosomes in tsetse with the aim of disrupting the transmission cycle and so provide additional options for trypanosomiasis control (Rio *et al.*, 2004).

##### **1.2.2.6.1 The life cycle and development of trypanosomes in tsetse**

The life cycle of pathogenic trypanosomes (*T. brucei* s. l., *T. congolense*, *T. simiae* and *T. vivax*) involves both vertebrate and tsetse fly hosts as illustrated in Figure 1.2.



**Figure 1.2: Life cycle of *Trypanosoma brucei*** (Source: Centers for Disease Control)

Tsetse flies ingest infective bloodstream trypomastigotes when they feed on an infected vertebrate host in whose circulation these infective forms are present. Within the fly, ingested trypanosomes undergo cycles of development and multiplication involving different parts of the alimentary tract; the sites and the duration of development depend on the sub-genus or species of infecting trypanosomes (Roditi & Lehane, 2008).

*T. vivax*, representative of the sub-genus *Duttonella*, completes cyclical development exclusively within the mouthparts of tsetse in about 5-13 days (Bruce *et al.*, 1911 in Stephen, 1986; Lloyd & Johnson, 1924) and ingested bloodstream forms of this species which reach the fly gut are destroyed by digestive enzymes within a few days (Stephen, 1986). The short and relatively simple developmental cycle undergone by

*T. vivax* is seen as an adaptation to facilitate its transmission by tsetse and accounts for its usually high infection rate in tsetse (Stephen, 1986).

Cyclical development of *T. (Nannomonas) congolense* and *T. (Trypanozoon) brucei* in tsetse is more complex and involves two distinct stages which include establishment in the midgut, and subsequent migration to the mouthparts and salivary glands respectively (maturation). Prior to an infection becoming established, majority (~99%) of the ingested trypanosomes are killed in the fly. However, ingested bloodstream forms which survive this attrition differentiate into procyclic trypomastigotes and migrate from the gut lumen via the distal end of the peritrophic membrane to the ecto-peritrophic space where they establish a midgut infection.

Majority of established midgut infections remain confined to the ecto-peritrophic space and fail to mature until the death of the fly but a few infections progress to the maturation phase (Peacock *et al.*, 2012a). Maturation of a *T. brucei* infection involves reinvasion of the midgut lumen by procyclic trypomastigotes through the anterior region of the peritrophic membrane, and migration to the proventriculus and foregut where differentiation to epimastigotes takes place. Epimastigotes invade the salivary glands where they transform into infective metacyclics and multiply (Sharma *et al.*, 2008; Peacock *et al.*, 2012a). *T. congolense* migrates in a similar manner but ultimately invades the mouthparts where differentiation to epimastigotes and then to infective metacyclics takes place (Peacock *et al.*, 2012b). Once a fly

develops a mature infection, it usually remains infective until it dies (Welburn & Maudlin, 1999).

Only mature infections are transmissible and the proportion of midgut infections that develops into mature salivary gland or mouthpart infections has been termed the transmission or maturation index (Peacock *et al.*, 2012a). Mid-gut infections have however been known to disappear or occur as very light infections (Roubaud *et al.*, 1935, cited by Stephen, 1986) and it was suggested that they were destroyed by bacteria in the fly gut (Lloyd, 1930; Peel, 1962). Stephen (1986) also mentions that workers at the then West African Institute for Trypanosomiasis Research (WAITR) had detected strict *congolense* infections in which only tsetse mouthparts were infected.

#### **1.2.2.6.2 Detection and identification of trypanosomes in tsetse**

The first and most common method used for the identification of trypanosomes in tsetse was developed by Lloyd & Johnson (1924) and involves the dissection of tsetse mouthparts, salivary glands and mid-gut followed by microscopic examination of these organs for trypanosomes. Trypanosome species were identified or differentiated based on which of the dissected parts are infected; proboscis only, *T. vivax*, mid-gut and proboscis, *T. congolense*, mid-gut and salivary glands, *T. brucei* while mid-gut-only infections are regarded as immature *T. brucei* or *T. congolense* infections (Lloyd & Johnson, 1924). However, with this method, mixed infections involving more than one trypanosome species and those involving sub-species of the

various sub-genera cannot be identified. Despite these shortcomings, this method is still widely used in many epidemiological studies.

To overcome the shortcomings of the method of dissection and microscopy, several molecular techniques which show better sensitivity and specificity for identifying trypanosomes in both vertebrates and tsetse have been developed. PCR based detection of trypanosomes in tsetse began with the use of species-specific DNA probes (Gibson *et al.*, 1988; Kukla *et al.*, 1987) and later species-specific primers for amplification of trypanosome DNA sequences in tsetse (Masiga *et al.*, 1992).

But while these methods were very specific and improved the accuracy of identifications of trypanosomes in tsetse, they were not sensitive enough to detect a wide range of trypanosome species and with species-specific PCRs, at least 11 separate primer sets and reactions were required to test each sample for all pathogenic species and sub-species of trypanosomes. This led to the development of generic primers and PCR protocols which enabled the identification of all known human and animal pathogenic trypanosomes in two reactions (Cox *et al.*, 2005) and finally in a single reaction (Adams *et al.* 2004; Njiru *et al.*, 2004, 2005).

The commonly used generic PCRs for the identification of trypanosomes in both tsetse and vertebrate samples are based on the amplification of a region of the Internal Transcribed Spacer 1 (ITS-1) of ribosomal DNA which shows sufficient inter-species variation to allow the differentiation of the known trypanosomes

(Adams *et al.*, 2006; Desquesnes *et al.*, 2001; Njiru *et al.*, 2005). Further species-specific PCR primers are however required to differentiate the sub-species of the sub-genus *Trypanozoon* which all produce the same band size with the generic ITS-1 primers (Njiru *et al.*, 2005).

These methods have been used successfully to identify trypanosomes in various species of tsetse from different parts of Africa (Adams *et al.*, 2006; Auty *et al.*, 2012) and are now used more frequently in epidemiological studies in many parts of Africa. They have been particularly useful in detecting mixed infections involving up to four different trypanosome species in a single tsetse fly (McNamara *et al.*, 1989, 1995; Solano *et al.*, 1995; Woolhouse *et al.*, 1996).

Nevertheless, there are still some limitations with using PCR to detect trypanosomes in tsetse. One major challenge is the difficulty of interpreting results especially with regard to the status of infections. Unless the tsetse flies were dissected and trypanosomes detected in the appropriate organs, it is difficult to determine whether the DNA detected by PCR represents a viable, transmissible infection. Another limitation is that not all trypanosome infections are identified for example; Adams *et al.* (2006) were only able to identify 56% of infections in dissection-positive tsetse caught in Tanzania.



#### **1.2.2.6.3 Tsetse refractoriness to trypanosome infections**

It has been observed that even in laboratory studies conducted under ideal conditions, many trypanosome infections, particularly those involving *T. congolense* or *T. brucei*, fail to become established in the tsetse midgut and that, a significant proportion of established infections do not mature (Peacock *et al.*, 2012a, 2012b). This implies that tsetse exhibit considerable resistance (refractoriness) to trypanosomes at different stages during their cyclical development and this is largely responsible for the low trypanosome infection rates often recorded in wild-caught and experimentally infected laboratory flies (Welburn and Maudlin, 1999).

Tsetse's first line of defense against ingested bloodstream trypanosomes was thought to be provided by the trypanocidal action of midgut lectins. These lectins were activated by blood serum and result in the death of majority of the ingested trypanosome population (Maudlin and Welburn, 1987). However, more recently, it has been postulated that antimicrobial peptides activated by the presence of other microbes in the early stage of trypanosome infections (Hao *et al.*, 2001) and reactive oxidant species (MacLeod *et al.*, 2007) might also be involved in tsetse refractoriness to trypanosome infections.

#### **1.2.2.6.4 Factors affecting the susceptibility of tsetse to trypanosomes**

Regardless of the method employed, it has been observed that trypanosome infection rates in wild tsetse are often very low; particularly with *T. brucei* and *T. congolense*. Even when tsetse flies are experimentally infected with these species of trypanosomes, a significant proportion fail to establish, and many established

infections fail to develop into mature infections. This has led to the conclusion that tsetse flies are naturally refractory to trypanosomes and in recognition of the importance of tsetse-trypanosome interactions in the epidemiology of the African trypanosomiasis, much research has been conducted to elucidate the factors affecting the susceptibility and refractoriness of tsetse to trypanosomes. These studies have revealed the multiplicity and complexity of the interactions between tsetse and trypanosomes that affect trypanosome infection rates in tsetse. Such factors include sex, species, genetic traits, teneral and nutritional state of tsetse, as well as the presence of bacterial endosymbionts in tsetse which play different roles in the fly's physiology (reviewed by Aksoy, 2003).

Fly age has been shown to an important factor affecting the infection of tsetse flies by *T. brucei* and *T. congolense* (Welburn & Maudlin, 1999). For example, experimental work shows that teneral tsetse (taking their first blood meal), and hungry flies are more susceptible to the establishment of midgut infections than non-teneral or non-starved flies due to a weaker immune response in the former (Kubi *et al.*, 2006). Male flies have also been shown to develop more mature infections than female flies particularly for Trypanozoon infections (Welburn *et al.*, 1995). Some species of tsetse e.g. *G. morsitans* are also more likely than others such as *G. palpalis* to develop a *T. brucei* or other trypanosome infection (Moloo and Kutuza, 1988).

#### 1.2.2.7 Endosymbionts and tsetse susceptibility to trypanosome infections

Like many insects with a restricted diet, tsetse flies are dependent on endosymbionts to synthesize the nutrients lacking in their vertebrate blood diet which they (tsetse) cannot synthesize. Three distinct bacterial endosymbiotic are known to occur in tsetse (Aksoy, 2003). They include an obligate primary (P)-symbiont *Wigglesworthia glossinidia*, a commensal, secondary (S)-symbiont *Sodalis glossinidius* and the parasitic *Wolbachia*. *Wigglesworthia* is confined within bacteriocytes which form a U-shaped bacteriome in the tsetse midgut (Aksoy, 1995). In contrast, *S. glossinidius* is found intra and inter-cellularly in different parts of the fly including the haemocoel, fat-body, head, and legs (Balmand *et al.*, 2013; Chen *et al.*, 1999) while *Wolbachia* parasitizes the ovaries and testes. *Wigglesworthia* and *S. glossinidius* are transmitted to the developing intra-uterine larva via maternal milk-gland secretions while *Wolbachia* is transmitted trans-ovarially (Chen *et. al.*, 1999; Ma and Denlinger, 1974 in Aksoy, 1995).

*W. glossinidia* is a member the Enterobacteriaceae and phylogenetic studies indicate that its association with tsetse is about 50-80 million years old (Aksoy, 1995) and it displays concordant evolution with its hosts (Cheng and Aksoy, 1999). Its genome has a size of about 700 kilobases and displays a streamlined functional capacity such that despite its relatively small size, it encodes a variety of vitamins and biosynthetic products that have been found to promote tsetse reproduction and nutrition throughout its life cycle (Akman *et al.*, 2002; Michalkova *et al.*, 2014). *Wigglesworthia* is present in all tsetse species and its absence renders female tsetse sterile, although this can be partially reversed by supplementation of the blood meal

with B-vitamins (Nogge, 1981). However, as this reversal is only partial, it is likely that *Wigglesworthia* has additional functional roles in tsetse which cannot be replaced by vitamin supplementation (Nogge 1981). Recent studies (Pais *et al.*, 2008; Weiss *et al.*, 2013) show, that the presence of *Wigglesworthia* during larval development crucially affects the ability of adult tsetse to mount an adequate immune response to trypanosome infections. Adult flies whose larval stages developed in mothers from which this symbiont had been eliminated with antibiotics produced a weaker immune response than their counterparts which emerged from *Wigglesworthia*-positive mothers (Weiss *et al.*, 2013).

The secondary symbiont of tsetse, *Sodalis glossinidius* also a member of the Enterobacteriaceae, is found exclusively in tsetse flies in which it resides both intra and inter-cellularly in the mid-gut, fat body and haemolymph (Balmand *et al.*, 2013). There is wide variation in the prevalence of *Sodalis glossinidius* in natural populations of tsetse and this indicates that this symbiont is not indispensable to tsetse.

However, there have been suggestions that this endosymbiont increases tsetse's susceptibility to midgut trypanosome infections (Maudlin and Dukes, 1986). Welburn *et al.* (1993) suggested that the increased susceptibility of *S. glossinidius* infected tsetse to midgut trypanosome infections was through its production of an endochitinase which degrades chitin during pupation and produces N-acetyl D-glucosamine; a sugar which inhibits trypanocidal midgut lectins. Later, MacLeod *et*

*al.* (2007) suggested that the N-acetyl D-glucosamine produced might function as an antioxidant which improves trypanosome survival in the tsetse midgut.

Research on wild caught tsetse has not been conclusive in terms of *S. glossinidius* playing a role in tsetse susceptibility to trypanosome infections. For example, Farikou *et al.* (2010) found a close association between the presence of *S. glossinidius* and trypanosome infections in *G. p. palpalis* from two HAT foci in Cameroon while Wamwiri *et al.* (2013) found only a weak association between the presence of this symbiont and trypanosome infections in *G. pallidipes* caught in Kenya. Contrary to these findings, Dennis *et al.* (2014) did not detect an association between *S. glossinidius* and trypanosome infections in *G. morsitans*, *G. pallidipes* and *G. brevipalpis* collected from Luambe national park in Zambia. With the development of techniques to produce in vitro cultures of *S. glossinidius*, research is focusing on using genetically engineered strains of this symbiont to produce less competent transgenic tsetse as another approach to controlling trypanosomiasis (Chen & Aksoy, 1999).

*Wolbachia* is a genus of endosymbiotic  $\alpha$ -proteobacteria infecting a wide range of arthropods and filarial nematodes ((Jeyaparakash and Hoy, 2000) and tsetse flies are among 20% - 70% of insects estimated to be infected by a variety of *Wolbachia* strains (Werren *et al.*, 2008). In many species as well as in tsetse, they cause several reproductive abnormalities including parthenogenesis, male-killing, feminization and cytoplasmic incompatibility (CI). Two basic forms of CI are recognised.

Unidirectional CI involves one *Wolbachia* strain in which matings between infected males and uninfected females results in the females producing fewer surviving offspring in comparison to other possible matings. This is due to an incompatibility between egg and sperm. Bi-directional CI is caused by two *Wolbachia* strains and can occur when mating partners are infected with different strains.

Different strains of *Wolbachia* occur in laboratory and natural populations of tsetse. In laboratory reared flies, an infection rate of 100% is often recorded but this varies widely in wild populations. For example, Doudoumis *et al.* (2012) studied *Wolbachia* infections in 3750 flies (551 specimens from six different laboratory stocks and 3199 from natural populations) of nine *Glossina* species from 10 African countries using a *Wolbachia* specific 16S rRNA-based PCR assay. *Wolbachia* was found to be prevalent in *G. m. morsitans*, *G. m. centralis* and *G. austeni* populations. It was also detected in *G. brevipalpis* and for the first time in *G. pallidipes* and *G. p. gambiensis* but not in *G. p. palpalis*, *G. tachinoides* and *G. f. fuscipes*; suggesting that the *palpalis* group flies are highly resistant to *Wolbachia* infections, although the reason for this is not yet known.

#### **1.2.2.8 Control of tsetse flies**

Problems of trypanocidal drug toxicity in humans and resistance in animals and the lack of suitable vaccines against trypanosomes makes control of the tsetse vector the most viable option for limiting the impacts of trypanosomiasis (Jordan, 1986). Tsetse fly control has thus been a central component in the management of animal and human trypanosomiasis and dates back to the colonial era during which epidemics of

sleeping sickness occurred in West and East Africa and many livestock were lost due to trypanosome infections (Ford, 1971). Many large-scale tsetse control campaigns were mounted at the time, with considerable success recorded for example, in north-east Nigeria, an area of about 220,000 km<sup>2</sup> was cleared of tsetse (Davies, 1971). However, following the withdrawal of the European powers from Africa, many of these programmes were not sustained and collapsed with the result that tsetse re-invaded previously cleared areas and incidence of human sleeping sickness and nagana began to increase in such areas (Schofield & Kabayo, 2008).

#### **1.2.2.8.1 Destruction of hosts and habitats**

The earliest methods used to control tsetse involved the destruction of large numbers of wild game (Swynnerton, 1912 in Jordan, 1986) and extensive clearance of vegetation (Morrison, 1946; Nash, 1948) to eliminate the food source and habitats of the flies. Later, the extent of vegetation clearance was reduced and only certain types of woody vegetation which constituted permanent tsetse habitats were cleared (Nash, 1948; Glover, 1965). Although these methods were quite successful in controlling tsetse, they were considered wasteful and ecologically unsound and were therefore, discontinued by the early 1950s and replaced by the use of insecticides which became the mainstay of tsetse control in Africa for over 60 years (Allsopp & Hursey, 2004).

#### **1.2.2.8.2 Insecticidal control of tsetse**

Insecticidal control of tsetse began with the application of organochloride insecticides: mainly dichlorodiphenyltrichloroethane (DDT) and dieldrin by ground

spraying over large areas of tsetse habitat (Allsopp, 2001). The persistence of these insecticides ensured their effectiveness against tsetse emerging from underground puparia several months after application but this meant that they could also affect non-target populations for longer periods (Allsopp & Hursey, 2004).

As the resting and breeding sites of tsetse later became better known, such sites were selectively sprayed; leading to significant reductions in the area that was treated and hence the volume of insecticide used (Davies, 1971). This effectively brought down costs and reduced negative environmental impacts. Ground spraying of vegetation inhabited by tsetse has been the most widely used insecticidal method against tsetse and is particularly suitable in areas with a prolonged dry season as it enhances the persistence of the insecticide. During this time, tsetse flies are under considerable environmental stress and concentrate in the most suitable microhabitats making them easier to target (Davies, 1964). Davies (1964) and (1971) provide detailed accounts of the successful application of this method for the control of *G. tachinoides*, *G. palpalis* and *G. m. submorsitans* in north-east Nigeria where an area approximately 220,000 km<sup>2</sup> was cleared of tsetse over a period of 10 years.

Eventually, insecticides were also applied from the air to vast areas of tsetse infestation and the sequential aerial technique (SAT) is one of these methods. The infested area is sprayed from the air usually at night using a non-residual, low dose, ultra-low volume insecticide (endosulfan) applied from the thermal exhausts of a fixed-wing aircraft equipped with powerful lights and advanced navigation systems



(Allsopp, 2001). The first application is expected to kill all adult flies while subsequent applications are directed at those emerging from underground puparia over a period of time. The timing of the applications is such that emerging adults are prevented from mating and producing new larvae. In most successful operations using this technique, five sequential treatments were applied at 15-20 day intervals (Allsopp & Hursey, 2004). This approach was used to eliminate tsetse from the Okavango Delta in Botswana (Kgori *et al.*, 2006)

In spite of the successes recorded using ground and aerial application of insecticides for tsetse control, a major shortcoming of these methods is that, sprayed areas are soon re-invaded by tsetse if the entire tsetse population is not eliminated or if there are adjacent infested areas (Davies & Bowles, 1979). In addition, there have been concerns over the impacts of using insecticides over large areas, on non-target organisms (Ormerod, 1976). Although these impacts have been shown to be minimal and reversible (Grant, 2001), ground and aerial spraying of insecticides for tsetse control is being scaled down in favor of methods in which insecticides are applied not to vegetation, but rather, to traps and targets made of cloth (artificial baits), or cattle (live baits) and tsetse are killed when they land on these objects (Vale & Torr, 2004).

#### **1.2.2.8.3 Traps and targets**

Control of tsetse flies using traps dates as far back as 1910 when black sticky traps worn by plantation workers were used to eradicate *G. palpalis* on the Island of Principe (Vale & Torr, 2004). Since then, many different traps made from blue and

black cloth in shapes and designs that mimic the visual perception of vertebrate hosts by tsetse have been developed and their efficiency has been greatly increased by the identification and deployment of odor-baits which attract more flies, and by the application of a synthetic pyrethroid (usually deltamethrin) to the trap cloth which kills alighting flies (Kuzoe & Schofield, 2004).

Targets operate in a similar manner to traps but are cheaper and simpler in design; comprising of a suspended screen of blue and black cloth impregnated with deltamethrin or other synthetic pyrethroid. Flies are attracted to the blue segments, land on the black segment and they are killed by the insecticide which can remain lethal to tsetse for up to a year when applied at concentrations of 0.6% w/v (Vale & Torr, 2004). The efficiency of targets is also increased by the addition of artificial odour baits such as acetone or octenol (Kuzoe & Schofield, 2004).

Cattle have also been used as live baits to control tsetse. This involves treating cattle with low concentrations of synthetic pyrethroids applied as a spray or pour-on thus creating a mobile target. It has been shown that the effectiveness of insecticide treated cattle for the control of tsetse rises with increasing cattle density and AAT but its success is dependent on the density of cattle in the tsetse infested area and on them being the main hosts of tsetse. Less insecticide can be used in a restricted application protocol (RAP) where only portions of the animals on which tsetse preferentially land and feed are sprayed and this has the advantage of reducing the cost of treatments (Torr *et al.*, 2007).

#### 1.2.2.8.4 Sterile Insect Technique

The sterile insect technique (SIT) is another method which has been used to control tsetse flies. It involves the rearing of large numbers of male tsetse which are sterilized by irradiation and then released into the wild population. When female tsetse mate are mated by these males, they are unable to produce viable offspring and this leads to the eventual collapse of the population. This method was used to eradicate *G. austeni* from the Island of Unguja in Zanzibar between 1994 and 1996 after about 8.5 million sterile males had been released (Vreysen *et al.*, 2000). In central Nigeria, SIT was used to eliminate *G. p. palpalis* from an area of about 1500 km<sup>2</sup> near Lafia, after the fly population had been reduced by 90% using biconical traps and insecticide impregnated targets (Oladunmade *et al.*, 1985; Takken *et al.*, 1986). However, the area was found to have been recolonized some 20 years after the operation was terminated due to lack of funds (Oluwafemi, 2008).

Successful tsetse control requires adequate area-specific knowledge of tsetse ecology. Information on tsetse distribution and density, population structure and hosts are of particular importance when deciding what methods to employ. As well, the cost, acceptability, sustainability ease of deployment and especially, sustainability of the chosen methods, are important determinants of success. The knowledgeable use of the available tools can certainly lead to the reduction and possibly, eventual elimination of tsetse populations across the African continent.

### 1.2.3 Mechanical Transmission of Trypanosomiasis

While the African trypanosomes can only be cyclically transmitted by tsetse flies, it is known that they can also be transmitted mechanically by Tabanids, *Stomoxys* species and other haematophagous dipteran flies. In contrast to cyclical or biological developmental or multiplicative process of the parasite does not occur in the vector; parasites are taken up with the blood during an interrupted feed, survive only a short period on the insect's mouthparts and are inoculated into a new host if the fly resumes feeding on another host quickly enough (Hoare, 1970 in Wells, 1972).

Mechanical transmission is often inferred where animal trypanosomiasis occurs in the absence of tsetse but its role in the epidemiology of trypanosomiasis in Africa remains highly controversial. In the past, proponents of this means of transmission argued that the low rates of human infective trypanosomes in wild tsetse could not solely explain the sleeping sickness epidemics that occurred and that in the absence of tsetse, the disease occurred in domestic animals (Duke, 1919 in Wells, 1972).

There have also been laboratory experiments to demonstrate the occurrence of mechanical transmission. Moloo *et al.* (2000) demonstrated experimentally, the mechanical transmission of *T. vivax* at a rate of 37.5% by interrupting the feeds of *G. m. centralis* on goats in Kenya. Neither *T. congolense* nor *T. brucei* was successfully transmitted in the same experiment and it was suggested that this was due to the low parasitaemia of these two species in donor goats whereas *T. vivax* parasitaemia was high. They concluded that under natural conditions, mechanical transmission is likely to occur only when trypanosomes are present in high numbers in the peripheral blood

of the infected host and Mihok *et al.* (1995) confirmed that when parasitaemia was high in donor blood, even *T. brucei* could be successfully transmitted by *Stomoxys* species.

To date, there is no consensus over the significance of mechanical transmission in the epidemiology of the disease although In field experiments under near-natural conditions, Desquesnes and Dia (2003a, 2004) demonstrated unequivocally, the mechanical transmission of *T. vivax* and *T. congolense* respectively in Burkina Faso using cattle and the Tabanid fly *Atylotus agrestis*.

So far, this literature review shows that much has been learned about tsetse and trypanosomiasis over the last 100 years. Tsetse flies continue to transmit trypanosomes on the African continent and remain a major limitation to rural development despite the destruction of their favoured habitats and wildlife hosts and the habitats where they live. However, as a consequence of human driven environmental change, tsetse flies have been forced into less suitable habitats and adapt if they can, as has been observed in the *palpalis* group, or face the threat of extinction as with the *morsitans* or savannah tsetse.

Tsetse distribution, abundance, genetics and interactions with trypanosomes and vertebrate hosts all play important roles in the epidemiology of the trypanosomiasis while the role of mechanical transmission by other biting flies remains obscure and debatable. Control of tsetse and the diseases they transmit are based on a

combination of old and new techniques which need to be deployed according to their suitability in different contexts and epidemiological settings. The subsequent and final sections of this review focus on the Jos Plateau in north central Nigeria and the tsetse and AAT problem there, which provides the main motivation for the investigations presented in this thesis.

#### **1.2.4 The Jos Plateau**

In the introduction to this chapter, brief mention was made of the changes that have taken place in the tsetse and trypanosomiasis status of the Jos Plateau within the last three decades. In this section, the Jos Plateau is described in some detail and particular attention is given to aspects of its climate, environment and ecology which are of relevance to tsetse and trypanosomiasis. This is followed by a review of the current state of knowledge on the epidemiology of trypanosomiasis in the area which provides the context and motivation for the investigations carried out in this thesis.

##### **1.2.4.1 Location and geology**

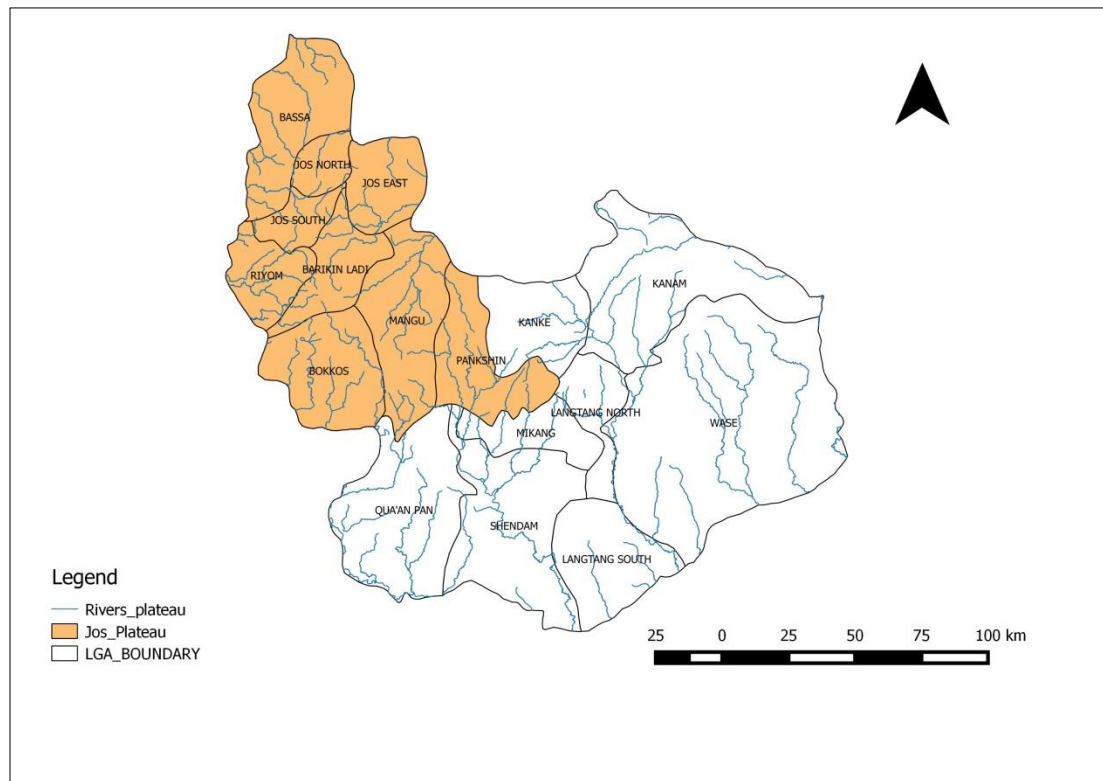
The Jos Plateau is situated in the northern part of Plateau State, which derives its name from this prominent geographical feature. Plateau State shares common boundaries with the States of Nasarawa, Kaduna, Bauchi and Taraba known to be tsetse-infested areas (Glover, 1965). Figure 1.2 is a map of Nigeria showing the location of Plateau State.



**Figure 1.3: Map of Nigeria Showing the Location of Plateau State.**

Source: (<https://plateaunewsonline.wordpress.com/>)

The area of Plateau State referred to as the plateau is located between latitudes  $8^{\circ}55'N$  and  $10^{\circ}11'N$  and longitudes  $8^{\circ}21'E$  and  $9^{\circ}30'E$  and comprises nine of the 19 Local Government Areas of Plateau State occupying an area of approximately 8600  $km^2$  (Phillips-Howard, 1992). It rises steeply from an altitude of about 200 m above sea level around the plains of the River Benue in the south, to an average height of 1280 m with peaks of up to 1829 m at Shere hills in Jos East (Alford *et al*, 1979). To the west and south, it is demarcated from the surrounding lowlands by a steep scarp 500 m to 700 m in height, but on the northern and eastern sides, the transition is less abrupt and it falls to the plains in a series of steps (WWF, 2014).



**Figure 1.4: Location of the Jos Plateau.**

The main rock type of the plateau is younger granite of volcanic origin and many of its extinct cones have crater lakes. Streams flowing from these lakes are the sources of several major Nigerian rivers like Kaduna, Gongola, Hadejia and Yobe and consequently, the Jos Plateau has been described as the hydrological centre for many of the river systems in northern Nigeria. Generally though, Plateau State itself is not well supplied with groundwater and the main affluents of the Benue River that cross the plateau; River Dep and River Mada, often dry up late in the dry season (Grove, 1952; Alford and Tuley, 1974).



#### **1.2.4.2 Climate**

The high relief of the Jos Plateau confers on it a generally cool climate and temperatures on the plateau are lower than the surrounding areas with minimum temperatures of between 15.5 °C and 18.5 °C and maxima of 27.5 °C to 30.5 °C. Average monthly temperatures are usually between 20 °C to 24 °C with maximum temperatures occurring in late March to early April while the lowest temperatures are recorded in December and January (Alford *et al.*, 1979).

Annual rainfall on the plateau ranges from 1000 to 1500 mm per annum and July and August are usually the wettest months. Due to its relief and the direction of air flow, the distribution of rainfall is not uniform across the plateau; the highest totals occur on the hilly western margin (particularly the south-western margin) and the lowest, on the eastern plains of Panyam and Pankshin adjacent to Bauchi State. The heavier rains in the south and west are due to the moisture-bearing south-westerly winds meeting the escarpment at this point (Alford *et al.*, 1979).

Two main seasons (wet and dry) are recognised on the plateau; governed by the seasonal migration of the Inter-Tropical Convergence Zone (ITCZ) (Alford *et al.*, 1979). The cool, dry months are often characterized by a dusty and dry north-easterly air flow resulting in clear skies and cold night temperatures, variable day-time temperatures, minimal to zero rainfall and dust haze (Harmattan) (Alexander, 1986). The climate of the Jos Plateau; notably its relatively low mean annual and minimum temperatures, has been cited as one of the main reasons why tsetse were absent from the bulk of its surface (Dede *et al.*, 2005; Pullan, 1980).

#### 1.2.4.3 Vegetation

The plateau falls within the Northern Guinea Savannah vegetation zone which is characterized mainly by open savannah woodland (Keay, 1953). The vegetation is dominated by savannah species, but also includes a number of species with South or East African affinities. The most numerous tree species in the remaining woodland is *Isobertia doka*, while *Vitex doniana*, *Lannea schimperi*, and *Uapaca somon* are also present (WWF, 2014).

Species commonly found along streams are *Syzygium guineense* and *Berlinia* sp. The following rock-loving species found in bushland and scrub forest on the plateau are also listed: *Carissa edulis*, *Dalbergia hostilis*, *Diospyros abyssinica*, *D. ferrea*, *Dodonaea viscosa*, *Euphorbia desmondii*, *E. kamerunica*, *E. poissonii*, *Ficus glumosa*, *Kleinia cliffordiana*, *Rhus longipes*, *R. natalensis*, *Ochna schweinfurthiana*, *Olea capensis*, *Opilia celidifolia*, and *Pachystela brevipes* (White, 1983). Many of these species are common to the lowland savannah areas surrounding the plateau and their presence on the plateau is thought to be due to the destruction of the original woodland of the plateau over at least several hundred years, and the presumed increased incidence of fires, which have allowed their spread to the plateau (White, 1983; WWF, 2014).

Due to the expansion of the human population and activities such as the extensive clearance of land for cultivation, mining, and collection of firewood and timber, the non-cultivated vegetation on most of the plateau comprises complexes of grass, shrub and sparse woodland (Alford and Tuley, 1974). Presently, only a few remnants

of woodland can be found on the plateau and these are restricted to its steep and less accessible south and western margins, with open grassland occupying the remainder of the plateau. Some fragments of forest occur on the southern and western escarpments, river banks, and along the base of rocky outcrops but even these are constantly being destroyed to make way for farmland. In and around villages, except for an occasional sacred grove, most trees are useful species such as the oil palm (*Elaeis guineensis*), canarium (*Canarium schweinfurthii*) and mango trees (*Mangifera indica*), many of which are planted and natural trees are largely to wholly absent in most areas (WWF, 2014).

#### **1.2.4.4 Human population**

At the last national census conducted by the Nigeria Population Commission (NPC) in 2006, the population of Plateau State was 3.2 million people with about 63% of this number residing in the nine local government areas (LGAs) that make up the Jos Plateau (NPC, 2006). This makes the Jos Plateau a densely populated area with about 200 to 300 people per square kilometer.

There are over 53 indigenous ethnic groups in Plateau State but overall, the population is cosmopolitan and there are hardly any of Nigeria's ethnic groups that are not found in the State. The ethnic composition and diversity of Plateau State is due to immigration of people from throughout Nigeria from the early colonial period; stimulated at the time by industrial-scale tin and columbite mining on the plateau which led to the establishment of Jos town and many smaller mining settlements inhabited mainly by people from other parts of Northern and Southern Nigeria

(Awogbade, 1983; Blench 2003). The Fulani, who are mainly pastoralists, are among those who migrated to the plateau although for reasons other than mining (Awogbade, 1983) and are discussed in a separate section because of their central role in cattle production and the associated problem of bovine trypanosomiasis on the Jos Plateau and in most of Nigeria.

#### **1.2.4.5 Agriculture**

Mining was the most important economic activity on the plateau during the colonial era but this has been replaced by agriculture which includes both arable farming and livestock production (Blench, 2004). The relatively fertile soil and favourable climate of the plateau make intensive farming with short fallow periods possible (WWF, 2014). Historically, cultivation was once limited to villages due to intertribal wars with large areas of uncultivated land separating adjacent villages. Later when intertribal disputes became less frequent farming activities were no longer concentrated around the villages. The areas of unfarmed, tree-covered land are now greatly reduced and very little land remains that is not affected by cultivation.

The soil and cool climate of the plateau make it suitable for the cultivation of exotic crops such as apples, potatoes, grapes, wheat, barley and vegetables and about 200 000 tons of potatoes are produced annually (OnlineNigeria, 2003). Most of the land is cultivated all-year-round and with the availability of water for irrigation derived from mine ponds, streams, and dams, over 40,000 hectares of land have been brought under irrigation in the last few years (OnlineNigeria, 2003). The expansion of cultivation has been facilitated by the availability of irrigation pumps, tractors, and

inorganic fertilizers and is driven by the need to meet the growing demand for food as local and national populations increase (Blench, 2003). Livestock on the plateau are mainly poultry, sheep, goats, pigs, dogs and cattle with a few donkeys and horses. The plateau is a major cattle production area with an estimated cattle population of about 1.05 million; 90% of which are owned by Fulani pastoralists (RIM, 1992).

#### **1.2.4.6 Fulani Pastoralism on the Jos Plateau**

The Fulani are the best known and most numerous of several pastoralist groups found in Nigeria (Blench, 1994) and it is estimated that they first arrived the far north of Nigeria having migrated from Senegambia between the 14<sup>th</sup> and 16<sup>th</sup> centuries (Awogbade, 1983; Blench, 1994; Stenning, 1957). For a long period after their arrival, they were confined to the semi-arid north of the country seemingly due to the risk of tsetse-transmitted trypanosomiasis to their susceptible zebu cattle in the more humid southerly zones (Blench, 1994). They, however, undertook annual southerly migrations during the dry season in search of grazing and water for their cattle; returning northwards as quickly as possible with the first rains which led to the expansion of tsetse populations in these southerly grazing areas (Glover, 1965).

Over time however, the Fulani gradually began to expand southwards within the country (Blench, 1994). Reasons given for this southward expansion include: increasing aridity and drought, reduced access to grazing due to expansion of the human population and intensive agriculture in the semi-arid north, and the Rinderpest outbreak of 1887 to 1894 (Stenning, 1957). Fulani expansion was also seen to have been facilitated by the Jihad (holy war) led by Usman dan Fodio, a

Fulani Muslim cleric between 1804 to 1830, and the intervention of the British colonial government which pacified the warring tribes of the northern and central States between 1902 and 1908 (Awogbade, 1983; Blench, 1994). In addition, the general reduction in tsetse populations in the central and southern parts of the country and increased availability and access to modern veterinary drugs meant that cattle were at less risk of trypanosomiasis in these areas. As such, Fulani and their cattle can now be found as far as Cross-River State in the south-east and Oyo in the south west of Nigeria (Blench, 1994).

The extensive grasslands, cool climate, and relative absence of tsetse and mosquitoes on the Jos Plateau, made it a very attractive destination for the Fulani who moved to the area below its escarpment in search of grazing for their cattle in the late 19<sup>th</sup> and early 20<sup>th</sup> century (Awogbade, 1983). But early attempts by the Fulani to settle on the plateau met with stiff resistance from the indigenous tribes and resulted in several constant warring and cattle raiding during most of the 19<sup>th</sup> century (Awogbade, 1983). Following the discovery of vast deposits of tin on the plateau, the British colonial government enforced peace between the warring parties around 1906 putting an end to the raids and opening up a way for the Fulani on to the plateau. Consequently, between 1908 and 1911, the first Fulani settlers and their cattle entered Miango (Bassa LGA) on the western escarpment of the Jos Plateau and eventually, spread to other parts of the plateau (Awogbade, 1983). For many decades after, they lived alongside the indigenous peoples who were mainly cultivators with minimal friction (Blench, 2003; Hickey, 1978). Fulani from Bauchi and other northern States have also settled on the plateau (Blench, 2003) and it is estimated that

about 300,000 Fulani currently live on the Jos Plateau and own some 1.05 million cattle which represent 90% of the cattle population in the area and ~7% of the national herd (RIM, 1992).

The Fulani practise an extensive system of production that depends mainly on the availability of natural grassland and pasture with little external input except for veterinary drugs (Awogbade, 1983). There is no system of supplementary feeding and so cattle are herded from place to place in search of grass and water. Though the majority of Fulani on the plateau are now settled agro-pastoralists who have permanent homesteads and grow crops for subsistence, they continue the traditional extensive system of cattle management with a semi-nomadic or transhumant lifestyle (Majekodunmi *et al.*, 2014).

During the wet season (May-September), most herds are grazed on communal pastures usually within a few kilometres from homesteads but during the dry season (October-March) when these pastures are depleted, the herds are trekked farther to low lying areas surrounding the plateau where there is more grass and water in a seasonal transhumance (Awogbade, 1979, 1983; Blench, 2003; Majekodunmi *et al.*, 2014). Recent observations show that in addition to the dry season migration, many herds now spend a considerable portion of the wet season off the plateau because the available grazing on the plateau can no longer sustain the large cattle population (Blench 2003; Majekodunmi *et al.*, 2014).

Indeed, Pullan (1980) had recognised the problem of seasonal food shortages for cattle on the plateau and at the time, he considered it to be a more important limitation to cattle production than trypanosomiasis which was very rare. The impact of the shortage of pasture on the plateau is clearly more severe at present due to a significant expansion in intensive small-holder agriculture on the plateau and a shift in the types of crops produced (Blench, 2003).

In the past, most farmers grew millet, sorghum and 'acha' (fonio) and after the harvest, the residues were available as browse for Fulani cattle in the early dry season and the farmers were in turn rewarded by the manure from the cattle. In recent times, maize, potatoes and other vegetables such as cabbage, lettuce and tomatoes account for more than 90% of the crops grown on the plateau; the residues of which are not useful to cattle. Almost year-round cultivation of these crops on once fallow land that used to be grazed by Fulani cattle and on the banks of streams and rivers where cattle are watered has been greatly facilitated by increased access to irrigation pumps, organic fertilizers and chemical pesticides (Blench, 2003).

With increasing pressure on land resources, the potential for conflict between herders and crop farmers is greatly increased and several violent conflicts have occurred between the Fulani and indigenous tribes in many areas of the plateau; especially, but not limited to the Berom in Barkin Ladi and Riyom and the Ron-Kulere people of Bokkos with whom they once lived in mutual dependence and cooperative exchange of goods and services. These conflicts over resources are exacerbated by the lingering ethno-religious and political conflict on the plateau between indigenes of



the plateau who are predominantly Christian and the Hausa and Fulani settlers who are mostly Muslims (Ostien, 2009). Higazi (2011) provides a detailed account of the protracted conflict on the plateau which has led to considerable loss of life and property and discusses possible reasons for these conflicts which are not limited to competition over land resources.

A major effect of the lingering conflict on the plateau on Fulani pastoralism appears to be an increase in the extent and frequency of Fulani migrations. In some areas of Barkin Ladi and Riyom, Fulani have been evicted, even from land which they had purchased, while in other areas where they are allowed to remain, they often have to move their cattle away during the wet season to avoid damage to crops which could lead to outbreaks of violence (Blench, 2003). Many of the evicted Fulani have moved to low-lying areas of Plateau State such as Kanam and Wase which host more sympathetic, Muslim populations while others have moved as far as the neighbouring States of Bauchi and Kaduna (Blench, 2004). Some of the displaced Fulani from the plateau have reportedly settled around forest reserves in Bauchi such as the Yankari Game Reserve on the border with Plateau State (Blench, 2004) where there is abundant grazing because of its protected status (Omondi *et al.*, 2006). These increased and often forced movements to areas like the Yankari Game Reserve which hosts an abundance of tsetse flies and wild game (Clement *et al.*, 2013; Omondi *et al.*, 2006), could expose cattle to trypanosome infections which are brought back to the plateau when they return and may be part of the reason why animal trypanosomiasis has increased in prevalence and spread on the Jos Plateau.

### 1.2.5 Animal Trypanosomiasis on the Jos Plateau

Animal trypanosomiasis was first recognised as an emerging threat to livestock on the Jos Plateau in the early 1980s and reports by Joshua (1982) and Joshua & Shanthikutmar (1989) represent some of the earliest records of the disease among sedentary stock on the plateau. Later, Kalu (1991) reported an outbreak of trypanosomiasis which occurred in 1987 among Fulani cattle in Bass during which a prevalence of 38.6% (95% CI: 34.8 – 42.5%) using the haematocrit centrifugation technique (HCT). Most of the infections (67%) were due to *T. vivax* while the remaining infections were unidentified. The outbreak had caused a 50% reduction in milk yields and the death of several animals. Animals found to be infected were treated with diminazene aceturate at the recommended dose of 3.5 mg/kg of body weight and uninfected animals received prophylactic doses of isometamidium chloride. Twenty-eight *G. tachinoides* were trapped in riverine vegetation in the locality and three (10.7%) harboured mature *T. vivax* infections.

Subsequent studies in several locations on the plateau (Anosike *et al.*, 2003; Kalu, 1996a, 1996b; Kalejaiye & Omotainse, 2000; Kalejaiye *et al.*, 2004; Qadeer *et al.*, 2008, Yanan *et al.*, 2007) employing various parasitological techniques, revealed trypanosome prevalence rates ranging from 5% to 40% in cattle mostly, but occasionally in sheep and goats with *T. vivax* often accounting for the majority of trypanosomal infections. *T. congolense* and *T. brucei* usually occurred at significantly lower rates.

In a recent longitudinal study of AAT on the plateau, Majekodunmi *et al.* (2013) sampled 7142 cattle from 30 villages spread across the plateau and found an overall

prevalence of 46.8% (CI: 39.0% - 54.5%) using species-specific PCR assays to screen blood samples for trypanosomes. *T. congolense* was the most prevalent trypanosome species and accounted for 27.7% of infections while 26.7% and 3.2% of the animals were infected with *T. vivax* and *T. brucei* respectively. This overall pattern was maintained for most part of the year except for the late wet season when *T. vivax* occurred in a higher percentage (29.9%) of animals compared to *T. congolense* (25.6%).

The dominance of *T. congolense* in addition to the overall high prevalence of trypanosome infections detected in the above study seems to indicate a change in the epidemiology of the AAT on the Jos Plateau. Alternatively, the study may have brought to light a situation that had not been detected in previous studies which were limited to few villages, had smaller samples and relied on microscopy for trypanosome detection as opposed to the higher number of villages, larger sample size and more sensitive PCR techniques used in this latest study. The study also found wide variations in the prevalence of infections between villages; the lowest prevalence was 8.8% and the highest was 95.6%. This wide difference is suggestive of spatial variations in trypanosomiasis risk probably due to differences in tsetse density and distribution. Cattle kept at the highest altitudes on the plateau had 35% the risk of those kept at lower altitudes and animals that migrated were 1.2 times more likely to be infected than those that did not (Majekodunmi *et al.*, 2013).

### **1.2.6 Tsetse Flies on the Jos Plateau**

In many studies of tsetse on the Jos Plateau (most of which were conducted from 1980 onwards), it is stated that the plateau was tsetse-free until the 1980s. However,

this assertion cannot be accepted as being correct in view of earlier studies; for example, Taylor (1930) and Nash (1948) had reported the presence of tsetse flies in Ganawuri and Rukuba both of which are less than 30 km from Jos, the capital of Plateau State.

Taylor (1930) described a severe outbreak of human sleeping sickness in Ganawuri situated at an altitude of about 950 m on the southern escarpment of the plateau. It was thought that unrestrained movement between different tribes in the area following improved communications, led to the introduction of new and virulent strains of *T. b. gambiense* into the locality where *G. p. palpalis* occurred and was incriminated as the transmitting agent. Game animals were rare because of the hunting and arable farming practices of the local population and the flies fed mostly on the human population and the domestic animals which included sheep, goats and dogs (Taylor, 1930).

Major concentrations of the fly were found at watering points on moderately shaded streams frequented by the villagers and their animals while a few flies occurred in heavy fringing forests with high shade at stream crossing points. They were restricted to heavily shaded portions of the streams during the dry season but often dispersed into the villages during the wet season. The epidemic was controlled by treating the people and relocating them from the hills to the nearby plains which were free of tsetse but no control activities were carried out against the vector population. Taylor observed that tsetse flies on the plateau were generally scanty because suitable heavy shade and an adequate food supply were comparatively rare but noted that *G.*

*palpalis* was present where conditions permitted (Taylor, 1930). These conditions were not stated but it is assumed that they would have included suitable habitat in the form of riverine vegetation and the availability of hosts.

The second historical record of tsetse on the plateau pre-1980 is found in Nash (1948) cited by Glover (1965). It refers to a sleeping sickness survey conducted in Rukuba on the Plateau's western escarpment (not far from Ganawuri) in 1941. Infection rates for *T. b. gambiense* ranged from 4% to 53% in surveyed villages and there were six deserted villages at the edge of the escarpment, whose inhabitants were said to have been killed by sleeping sickness. *G. p. palpalis* was detected in stream vegetation but the flies were reportedly eliminated from the area by ruthless clearance of vegetation from the tsetse infested streams.

Thirdly, Sangree (1970) in a study of the relationship between ritual leadership and mortality rates among the Irigwe who lived in Kwall and Miango just above the western escarpment of the plateau, refers to a study by Moir (1933) on sleeping sickness among the people of Kwall and Miango in which it was reported that *G. p. palpalis* was present in forest patches designated as sacred groves and bit men who visited the groves to perform ritual sacrifices. Sangree (1970) concluded that mortality among this group of men that the local people attributed to an offence against the 'gods', was probably due to tsetse-transmitted sleeping sickness.

A fourth reference to tsetse presence on the plateau pre-1980 can be found in Tenabe & Greiling (1981) who reported on parasitism by *Sytomosphyrum glossinae* within a

colony of *G. palpalis* maintained at the Biological Control Project for Tsetse flies (BICOT) laboratory located in Vom close to Jos. The colony was established from puparia supplied by the International Atomic Energy Agency (IAEA) in Vienna and the flies were used in a trial of the sterile insect technique (SIT) as a tsetse control option in Lafia (now in Nasarawa State) south-west of the plateau. The authors concluded that the parasitoid *Sytemosphyrum glossinae* was introduced into the colony by *G. palpalis* puparia collected from Kurra falls in Barkin-Ladi on the plateau during the previous dry season.

All of the above accounts clearly illustrate, that *G. p. palpalis* was present on the Jos Plateau as early as 1927; negating claims that the plateau was tsetse-free prior to the 1980s and only invaded thereafter. It is likely that the invasion of the plateau by tsetse occurred as early as 1908 when Fulani pastoralists and their cattle first settled in Miango on the western escarpment of the plateau. The fact that human sleeping sickness occurred in Ganawuri, Miango and Rukuba (all in the same general area), provides some support for such a view. Subsequent studies of tsetse on the plateau have mostly been in connection with animal trypanosomiasis and commenced in the 1990s; perhaps as AAT became increasingly prevalent.

#### **1.2.6.1 Species, distribution and abundance of tsetse on the Jos Plateau**

*Glossina palpalis* and *G. tachinoides* are the two main species of tsetse recorded on the Jos Plateau (Abbah *et al.*, 2011; Dede *et al.*, 2005; Kalu, 1996a, 1996b; Majekodunmi, 2006). However, in one study, Onah (1995), the presence of *G. m. submorsitans* alongside *G. palpalis* and *G. tachinoides* was recorded in a cattle

market in a densely populated area of Jos, where there was no natural tsetse habitat. It was suggested that the flies were transported into the area on trucks bringing cattle to this market from Bauchi, Adamawa and Taraba States. The flies rested in the shade of the market stalls and obtained blood meals from the trade cattle which provided an abundant and easily accessible food supply. There have since been no further reports of *G. morsitans* in this market or anywhere else on the plateau.

It is interesting to note that tsetse on the plateau, especially *G. palpalis*, have been reported mostly from villages in Bassa, Riyom, Barkin Ladi and Bokkos located on the southern and western escarpments of the plateau. *G. tachinoides* has also been reported in Kwall and Binchin of Bassa LGA again on the west, but also in Federe in Jos East LGA on the eastern escarpment where it occurred in both riverine and peri-domestic habitats (Dede *et al.*, 2005). This limitation of tsetse to the south and west escarpment would further support the theory that invasion of the plateau by tsetse took place along the south west escarpment and the flies have not really been able to spread to the central areas of the plateau which are densely populated and have been stripped of riverine vegetation which could have provided suitable habitat for the flies.

Reported apparent densities of tsetse in the few areas where they have been studied on the plateau are between 2.5 to 4.6 flies per trap per day (Kalu, 1996a, 1996b; Dede *et al.*, 2005; Majekodunmi, 2006).. These relatively low densities could be due to the scarcity of suitable riverine habitats and the fragmentation and destruction of

those that remain by the common practice of clearing riverine vegetation in order to grow irrigated vegetables on stream and river banks.

#### **1.2.6.2 Trypanosome species and infection rates in tsetse on the Jos Plateau**

There is very little information on the species and infection rates of trypanosomes in tsetse flies on the plateau. Dede *et al.* (2005) reported an overall infection rate of 1.67% in *G. palpalis* due to *T. brucei* and *T. vivax* but did not indicate the exact proportion of each trypanosome species. Kalu (1991) recorded three mouthpart-only infections likely to be *T. vivax* among 28 *G. tachinoides* and in another study in the same area, Kalu (1996a) found that one of nine (11.1%) trapped *G. tachinoides* harboured *T. vivax* infection while none of five *G. palpalis* dissected were infected. In a separate study in the same area,

In these studies, it is unlikely that all the flies were uninfected as a result of being newly emerged or unfed (teneral), as on dissection, many were found to be gravid (Dede *et al.*, 2005) and the mean age determined to be  $23 \pm 1.5$  days (Kalu, 1996a). The low infection rates may have been due to the absence of infective hosts in the area or the resistance of the vectors to acquiring trypanosome infections. The low sensitivity of the method (dissection and microscopy) used to detect trypanosomes in the flies could also be responsible for the results. Nevertheless, the prevalence of trypanosomes in cattle sampled during some of these studies was quite high e.g. 38.6% in Bassa (Kalu, 1991) and suggests either a high rate of transmission by few infected vectors or that the animals had acquired infections elsewhere where tsetse were more abundant and had higher rates of trypanosome infection.



Majekodunmi (2006) used both microscopy and molecular techniques (ITS-PCR) to identify the trypanosomes in tsetse from Assop, Kadunu some villages of the plateau. The overall trypanosome prevalence among 140 tsetse flies (31 *G. palpalis* and 109 *G. tachinoides*) was 40.7% (95% CI: 33.1 – 50.0%). Several species of trypanosomes were detected and included: *T. congolense* (11.8%), *T. brucei* (10.3%), *T. vivax* (8.8%), *T. godfreyi* (27.9%) and *T. simiae/simiae* Tsavo (41.2%). This was the first and only study to identify more than one species of trypanosome in tsetse on the plateau and this could be due to the more sensitive technique of trypanosome detection employed. The trypanosome infection rates were also unprecedented and it was suggested that there may have been misidentification of sperms as trypanosomes or cross contamination between samples during dissection due to the researcher's inexperience in carrying out tsetse dissections (Majekodunmi, 2006).

The above studies indicate that there is much variation in the trypanosome species and their infection rates in tsetse on the plateau and additional studies of trypanosomes in tsetse on the plateau using molecular techniques could provide more clarity on the trypanosomes circulating within the vector population in this area.

### **1.2.7 Tsetse and Trypanosomiasis control on the Jos Plateau**

There is currently no centralised or government-led tsetse control programme on the plateau and there is no information about any deliberate attempts to control tsetse in this area in the past. Autonomous control of tsetse due to the destruction of their natural habitats as land is cleared for farming especially on stream and river banks is probably the only form of tsetse control on the plateau. Perhaps if human sleeping

sickness remained endemic on the plateau, there may have been more efforts aimed at controlling tsetse.

The lack of organised tsetse control programmes in the area may also be an indication that AAT is not viewed by the relevant authorities as a problem requiring urgent attention. Consequently, livestock owners on the plateau depend on the use of curative (diminazene aceturate) and prophylactic (isometamidium chloride) trypanocides to treat and protect their animals against trypanosomiasis (Majekodunmi *et al.*, 2013). It has been observed that most of these drugs are purchased from drug vendors, and are sometimes of dubious quality; besides which they are administered without proper adherence to recommended dosage instructions (Kingsley, 2014; Majekodunmi *et al.*, 2013). These practices could lead to the emergence of drug resistant trypanosome strains although this has not been investigated or reported in the area.

The information on tsetse and trypanosomiasis on the Jos Plateau provided by the studies reviewed in this section can be summarised as follows:

1. Animal trypanosomiasis is currently endemic and widely distributed on the plateau with wide variations in prevalence, which might be explained by the differences in methods, timing, and location of studies but could also be due to variations in tsetse presence/density which have not been demonstrated. Most affected by AAT are transhumant herds of zebu cattle owned by settled Fulani agro-pastoralists.

2. Most studies show *T. vivax* to be the most common trypanosome in the area but recent work suggests that *T. congolense* could be as prevalent or even more so. This could be an indication of a change in the epidemiology of the disease due to increased involvement of tsetse flies in transmission. Mechanical transmission by biting flies is also thought to be occurring but remains unconfirmed.
3. Most studies frequently state that tsetse flies only invaded the plateau in the 1980s which coincides with the emergence of animal trypanosomiasis in the area. However, studies pre-dating the 1980s confirm that tsetse flies were present at least since the 1920s and so AAT probably gained prominence only as more Fulani settled in the area and cattle numbers increased; bringing cattle into more frequent contact with established tsetse populations.
4. *G. palpalis* and *G. tachinoides* are the two main species of tsetse found on the plateau, with *G. palpalis* occurring mainly around its west and southern edges (Bassa, Riyom, Barkin Ladi, and Bokkos) while *G. tachinoides* occurs mainly along the eastern margin (Jos East LGA) but also in Bassa on the western escarpment. Previous tsetse surveys have been confined to few villages and their results may not represent overall distribution and abundance of tsetse across the plateau.
5. The low density of tsetse, coupled with generally low trypanosome infection rates would suggest a low transmission risk and as such, it is difficult to

explain the high trypanosome prevalence sometimes recorded in cattle in the area. It is possible that other sources of infection (mechanical transmission or introduced infections from outside the plateau) are involved.

6. The seasonal migration of cattle across and away from the plateau in search of grazing and water (transhumance) is indicated as a possible risk factor for trypanosomiasis in the area. These movements are thought to bring cattle in contact with tsetse flies but no studies have recently investigated the presence of tsetse or the transmission of trypanosomes to cattle along migration routes or at final destinations.
7. There is no government involvement in tsetse and trypanosomiasis control and livestock owners purchase and administer curative and prophylactic trypanocides to their animals often without adhering to instructions on dosage; a practice that could lead to the emergence of drug resistant strains of trypanosomes.

### **1.3 Stamp Out *Sammone* (SOS) and the Current Study**

From the above summary, it is evident that bovine trypanosomiasis is established and occurs widely on the Jos Plateau with no government involvement in control of the disease and its vectors. This situation has negative consequences for the productivity of livestock and the livelihoods of their owners in the area and the need for appropriate interventions to mitigate these impacts is recognised (Majekodunmi *et al.*, 2013).

To address the current situation, and aid the development of a community-based strategy for AAT management on the plateau, a four-year research-for-development initiative tagged '*Stamp out Sammore*' (SOS) implemented jointly by the Nigerian Institute for Trypanosomiasis Research (NITR) and the University of Edinburgh, was launched in 2010. Funded by the Biotechnology and Biological Sciences Research Council (BBSRC) of the United Kingdom (UK) under its Combating Infectious Diseases in Livestock for International Development (CIDLID) scheme, it aimed to test the effectiveness of several options for the integrated control of trypanosomiasis and tick-borne diseases in cattle in six selected villages. Other activities under the project were socio-economic surveys to determine the impact of AAT on pastoral livelihoods and the willingness of livestock owners to participate in and sustain an integrated control project. The results of these are to be used to select a strategy for the control of trypanosomiasis most suited to the agro-ecological situation of the Jos Plateau pastoral herds.

Bouyer *et al.* (2013) have advocated that small-scale community-based interventions could be the most feasible management strategy for AAT in affected areas of Africa. They however stress that the effectiveness and sustainability of such interventions will depend on adequate understanding of the ecological and epidemiological context as well as the cattle management systems within which the disease occurs. Of particular importance in defining the epidemiological context of AAT, is adequate information on vector distribution, circulating trypanosomes within the vector and disease transmission routes or patterns; which for the Jos Plateau is currently patchy, incomplete and neEds. to be updated. It is against this background and within the

SOS project that the research presented within this thesis was conceived and conducted.

## **1.4 Thesis Aims and Objectives**

The main aim of this thesis is to provide additional understanding on the ecology of tsetse and the transmission of bovine animal trypanosomiasis on the Jos Plateau. It investigates the current distribution and density of tsetse in selected villages and examines the importance of seasonal cattle movements and mechanical transmission as alternative or additional sources of trypanosome infections in cattle on the plateau. The results are expected to inform the selection and deployment of appropriate control measures against AAT on the Jos Plateau. The study also provided an opportunity is to investigate for the first time, the prevalence of endosymbionts in wild tsetse from the Jos Plateau. The specific objectives of the study are:

1. To determine the species, distribution and apparent density of tsetse flies in selected villages of the Jos Plateau.
2. To determine the role of seasonal cattle movements in the transmission and epidemiology of animal trypanosomiasis on the plateau by investigating the presence of tsetse along migration routes and monitoring cattle for trypanosome infections during migration.
3. To identify the trypanosomes and endosymbionts present in tsetse trapped from the study villages and determine if there is an association between the

presence of *Sodalis glossinidius* and trypanosome infections in the trapped tsetse.

4. To assess the role of other biting flies ‘mechanical transmitters’ in the transmission of animal trypanosomiasis on the plateau.

## **1.5 Thesis Structure**

Following the introduction and literature review, Chapter 2 presents the results of two tsetse surveys; a longitudinal survey aimed at detecting spatial and temporal variations in tsetse distribution and apparent density in seven selected villages, and a cross-sectional survey conducted in 13 additional villages to provide a broader view of tsetse distribution across the plateau.

Chapter 3 examines the seasonal migration of Fulani cattle and its role in the introduction of infections of animal trypanosomiasis to the plateau. It does this by tracking the migration of a herd from the plateau, trapping tsetse along the cattle migration route and destination as well as monitoring a portion of the herd for trypanosome infections during one wet season (June-September 2013) and one dry season (October 2013-March 2014).

In Chapter 4, the prevalence of trypanosomes and endosymbionts in the tsetse flies captured during the surveys in Chapters 2 and 3 is investigated. Included also, are tsetse flies trapped in Yankari Game Reserve near which Fulani pastoralists avoiding

civil unrest on the plateau are reported to have established temporary camps. It is possible that such pastoralists will return to the plateau bringing cattle with new or more virulent strains of trypanosomes which would affect the epidemiology of the AAT in the area. The flies from Yankari were kindly provided by colleagues from the University of Jos, Plateau State, Nigeria.

Chapter 5 investigates the possibility of mechanical transmission of trypanosomes by *Stomoxys* spp. and Tabanidae by determining their relative abundance and examining their mouthparts and guts for the presence of trypanosome DNA using molecular techniques.

Chapter 6 discusses the major findings from the investigations in chapters 2 to 5 and their implications for the future of tsetse and trypanosomiasis on the Jos Plateau. It contains recommendations for integrated control of the disease and its vectors and also includes some suggestions for future research on tsetse and trypanosomiasis in the area.



## **2**

# **The Distribution of Tsetse Flies on the Jos Plateau**

## 2.1 Introduction

Tsetse flies, *Glossina* species, are distributed discontinuously over an area of approximately 10 million square kilometres in sub-Saharan Africa. They are responsible for the cyclical transmission of animal and human infective trypanosomes that cause AAT and HAT. These diseases can be fatal if left untreated and lead to substantial economic losses estimated at about US\$ 4.75 billion per annum (FAO, 2014). Consequently, the distributions of the various tsetse species are of particular importance to efforts aimed at alleviating poverty in Africa.

The limits of tsetse distribution are determined principally by climatic factors such as rainfall, temperature and humidity and these are influenced by altitude and vegetation which can mitigate the severity of climate (Nash, 1969; Rogers and Randolph, 1993). At the northern edge of their distribution, tsetse flies are limited by high temperature and dryness, whereas the southern limit seems to be determined by seasonal low temperatures (Rogers and Randolph, 1986). Altitude limits tsetse distribution by its effect on temperature, which is lower at higher altitudes. At the equator the altitudinal limit of tsetse is about 1800m although this decreases with increasing latitude (Glasgow, 1963).

Vegetation, which is dependent on climate, is also an important factor determining tsetse distribution as it provides shade and helps to maintain a suitable microclimate vital for tsetse survival (Leak *et al.*, 1999). It also serves as the habitat of the vertebrate hosts on which tsetse are dependent for blood meals. The importance

placed on vegetation as a determining factor of tsetse distribution is reflected in the grouping of the 31 species and sub-species into three broad categories on the basis of the main vegetation type in their preferred habitat. Thus, there are the savannah or *morsitans* group of tsetse, the forest or *fuscus* tsetse, and the *palpalis* or riverine tsetse flies which occupy the vegetation along water courses. At finer scales, the quality of the vegetation also affects tsetse distribution and density as seen in the significant positive relationship between *G. palpalis* abundance and the size of riverine forest patches (Bourn, 1983). In Burkina Faso, Bouyer *et al.* (2005) also found that fragmentation of riverine forests had a negative effect on tsetse presence and density.

In recent times, methods using remotely-sensed climate and vegetation data obtained by satellites have been developed for the prediction/modelling of tsetse distribution across the African continent (Hay *et al.*, 2000; Wint, 2001). Since there are spatial variations in the climatic factors and vegetation characteristics that determine tsetse distribution and abundance, it is expected that there will also be local variations in tsetse distribution. Determining these at the smallest spatial scales (e.g. at village level), and identifying specific local conditions responsible for such variations is important in designing area specific control programmes for tsetse and the diseases which they transmit. Hence, data on the presence/absence and density of tsetse derived from actual surveys at small spatial scales are still needed to improve the accuracy of predictive models.

The Jos Plateau, latitude 8°55'N - 10°11'N and longitude 8°21'E- 9°30'E (Phillips-Howard, 1992a), occupies an area of 8600 km<sup>2</sup> in north central Nigeria and was regarded as tsetse-free even though historical records show that *G. palpalis* had been reported on the plateau by the 1920s (Taylor, 1930) even though recent studies do not mention this. This is, however, attributable to the fact that the plateau has a high average altitude of about 1280 metres, and low average monthly temperatures (20-24°C) which were considered unfavourable for tsetse survival. Besides, trypanosome infections in sampled cattle at the time were so rare that Pullan (1980) concluded that trypanosomiasis was not a significant hindrance to cattle production on the Jos Plateau.

A few years later, however, studies demonstrated an increasing prevalence of trypanosomes in cattle on the plateau (Joshua, 1982; Joshua & Shanthikutmar, 1989) and thereafter, a couple of outbreaks resulting in significant morbidity and mortality were recorded (Kalu, 1991, 1996). Other studies over the years (Anosike *et al.*, 2003; Kalu, 1995, 1996; Kalejaiye & Omotainse, 2000; Kalejaiye, 2006; Qadeer *et al.*, 2008, Yanan *et al.*, 2007) have reported AAT prevalence rates ranging from 5-40% in domestic livestock; mostly cattle, in different locations on the plateau. Subsequently, entomological surveys also revealed the presence of tsetse flies (mainly *G. palpalis* and *G. tachinoides*) at low densities in some villages of the plateau (Dede *et al.*, 2005; Kalu, 1996a, 1996b; Majekodunmi, 2006).

In spite of the low density of tsetse recorded in these surveys, AAT is now endemic and widespread among Fulani-owned zebu cattle grazed extensively on the Jos

Plateau (Majekodunmi *et al.*, 2013). Based on a survey conducted in 2008, Majekodunmi *et al.* (2013) reported an overall trypanosomosis prevalence of 46.8% (CI: 39.0% - 54.5%) in 7142 cattle from 30 villages spread across the Jos Plateau. *T. congolense* was found to be most prevalent and accounted for 27.7% of infections while 26.7% and 3.2% of the animals were infected with *T. vivax* and *T. brucei* respectively. The high overall prevalence and dominance of *T. congolense* strongly suggested the involvement of tsetse flies in transmission at varying levels of challenge in individual villages. It is also an indication that tsetse might be more widely distributed on the plateau than was previously thought with the added possibility that there are seasonal changes in tsetse distribution and density as well as the frequency of contact between tsetse and cattle.

It is apparent from these studies that the AAT situation on the plateau has worsened over the last three decades with the current situation posing a significant risk to livestock health and productivity and the livelihoods of Fulani pastoralists who own most of the cattle on the plateau. Nevertheless, the factors influencing these changes are not yet fully understood. The increased occurrence of trypanosomiasis in livestock on the plateau is often attributed to the 'recent' presence of tsetse flies on the plateau as it is believed that tsetse only invaded the plateau in the 1980s. However, evidence from earlier studies suggests that this invasion must have taken place much earlier. For example, Glover (1930) and Nash (1948) described outbreaks of sleeping sickness on the plateau and confirmed the involvement of *G. palpalis* as the vector. It seems likely that AAT only gained prominence on the plateau with the

advent of Fulani pastoralists and their cattle which led to more frequent contact between cattle and tsetse.

Two species of tsetse (*G. palpalis* and *G. tachinoides*) have been found to occur mainly in riverine habitats on the Jos Plateau. However, knowledge of their distribution across the area remains limited as most past surveys have either been in response to outbreaks of trypanosomiasis in a single village or herd, or otherwise focused on single villages and involved a few days of trapping at a single time point. As such, they are quite limited in their coverage and provide no information on seasonal variations in tsetse distribution and density which may produce the observed seasonal patterns of disease prevalence. Therefore, obtaining up-to-date information on tsetse distribution especially in villages where AAT prevalence is high as well as in those where it is low or not known, is considered an important and necessary step in the design and implementation of effective tsetse and trypanosomosis control on the Jos Plateau.

## **2.2 Chapter Aim and Objectives**

The aim of this chapter is to provide information on the current distribution and density of tsetse flies in selected villages on the Jos Plateau and thus identify areas where there is a risk of trypanosomiasis transmission. It investigates tsetse presence and seasonal variations in tsetse density in seven villages. Thirteen other villages were sampled for tsetse in a cross-sectional survey to further determine if tsetse flies are widely distributed and whether the altitudes at which villages were located had an

effect on tsetse presence and density. The effects of temperature, rainfall and vegetation on tsetse presence and density are also explored and discussed. The specific objectives are:

1. To determine the species and density of tsetse flies in the study villages
2. To identify the main habitats of tsetse in the study villages including whether they occur in peri-domestic habitats outside of their preferred habitats.
3. To determine the occurrence of seasonal changes in the distribution and density of tsetse flies in the area.
4. To examine the effect of altitude, temperature, rainfall and vegetation on the presence of tsetse in the study villages.

## **2.3 Materials and Methods**

Two surveys were designed to meet these objectives: a longitudinal, and a cross-sectional survey in two sets of villages and are described below.

### **2.3.1 Longitudinal tsetse survey**

In order to study tsetse distribution and density and with the intention of detecting seasonal trends, seven villages were selected for a longitudinal tsetse survey conducted over an 18-month period from March 2012 to September 2013 (Table 2.1). The commencement of the survey (March/April 2012) coincided with the end

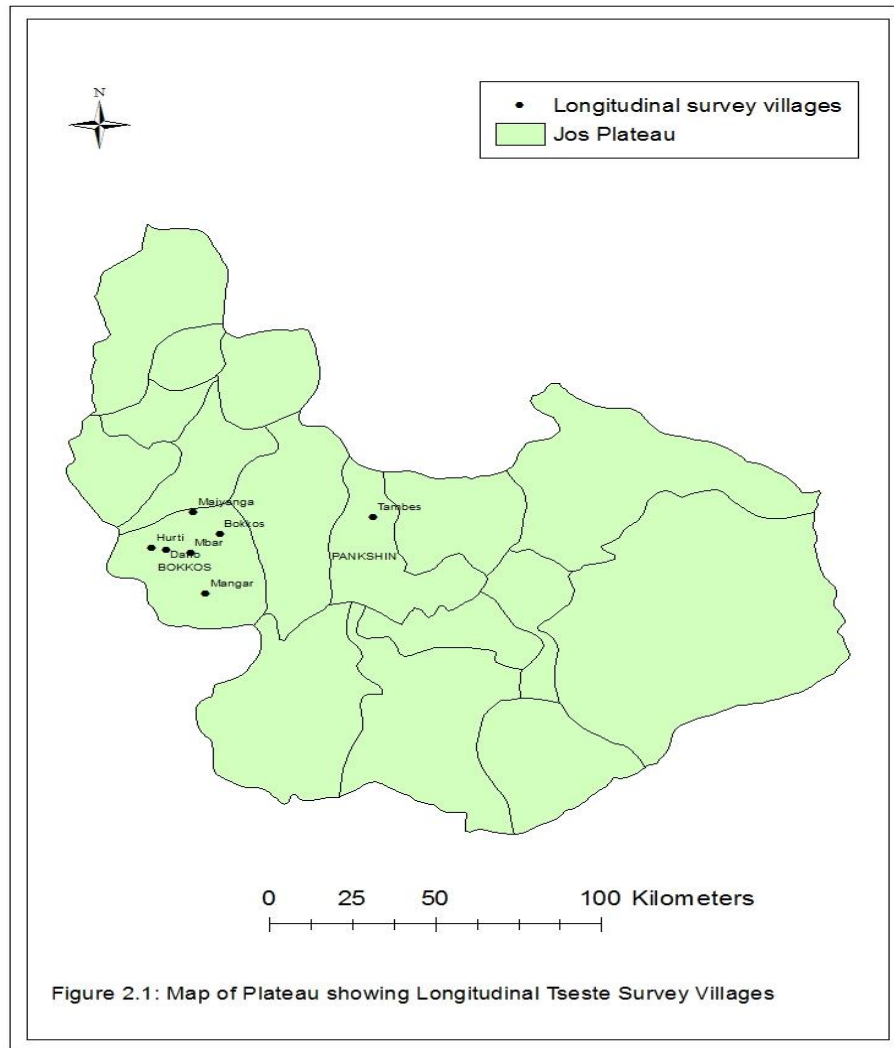
of the dry season, which began in October 2011 and the start of the wet season of 2012. The period of the survey therefore included two wet seasons and one dry season.

**Table 2.1: Trapping Schedule for Longitudinal Survey**

Trapping session	Period of sampling	Season
1	March/April 2012	Late hot dry
2	May/June 2012	Early cool Wet
3	June/July 2012	Mid cool Wet
4	July/August 2012	Mid cool Wet
5	September/October 2012	Late cool Wet
6	October/November 2012	Early Cool dry
7	November/December/2012	Mid Cool dry
8	December 2012	Late cool dry
9	February/March 2013	Mid Hot Dry
10	March/April 2013	Late Hot Dry
11	May/June 2013	Early Wet
12	June/July 2013	Mid Wet
13	August/September 2013	Mid-Late Wet

The villages for the longitudinal survey were chosen because of the high overall prevalence (20%-80%) of trypanosomiasis recorded in their cattle populations in 2008 (Majekodunmi *et al.*, 2013.) These villages include Maiyanga, Bokkos, Mbar, Mangar, Daffo and Hurti in Bokkos LGA on the south-western part of the plateau, and Tambes in Pankshin LGA in the south-east (Figure 2.1).





**Figure 2.1: Map of Plateau showing Longitudinal Survey Villages**

Climatic conditions in the villages are generally similar to those described for the Jos Plateau although small differences between villages are likely. There were varying amounts of what might be considered suitable tsetse habitat in each village. Dry season cultivation of potatoes and other vegetables on the banks of streams and rivers is a common feature of the six villages in Bokkos LGA but this practice was not observed in Tumbes in Pankshin LGA. Pesticides and inorganic fertilizers are

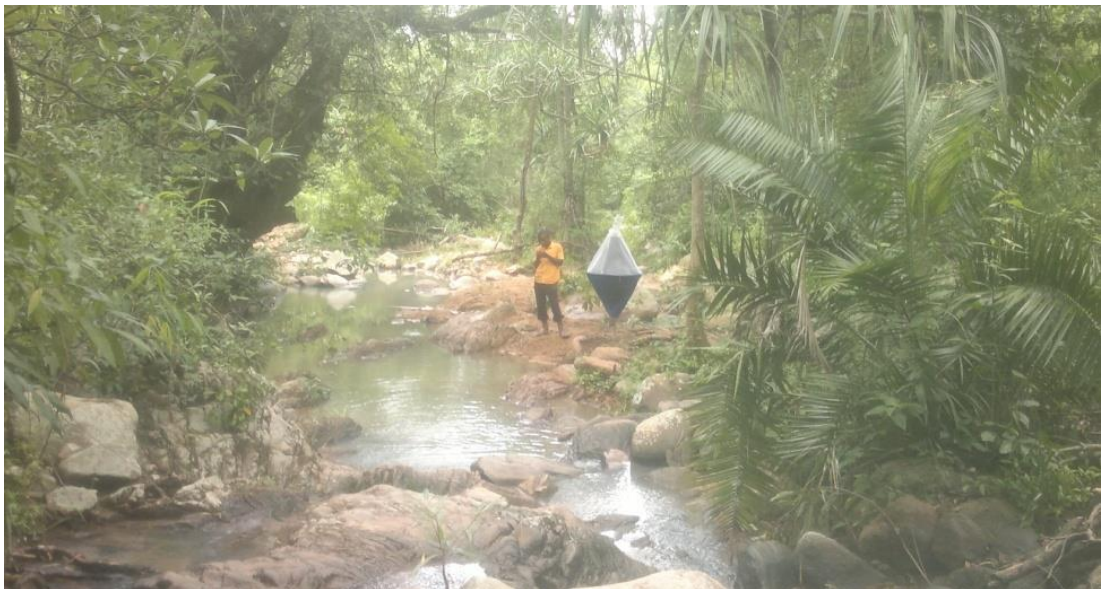
commonly employed in farming operations. Cattle are present in all villages and inquiries at the Nigeria Institute for Trypanosomiasis Research (NITR) in Vom revealed that none of these villages have previously been surveyed for tsetse. Some features of land use observed in the villages during sampling are provided in Table 2.2.

**Table 2.2: Land-Use in Villages selected for Longitudinal Tsetse Survey**

Village	Characteristics
Maiyanga	Cultivation intense and vegetation cover is very sparse; a few isolated thickets of <i>Lantana</i> . Very narrow, shallow streams with little vegetation. No wild animals seen.
Bokkos	Cultivation intense; dominated by farmland. Riverine vegetation occurs only in narrow strips often cleared at many points. Wide and relatively deep stream/river. No wild animals seen
Mbar	Cultivation intense; dominated by farmland with a narrow strip of dense riverine forest. No wild animals seen
Daffo	Cultivation intense; up to stream bank and in stream bed in dry season but there are wooded areas appearing to be remnants of previously more extensive riverine woodland. Monitor lizard seen
Hurti	Very rocky terrain with some dense vegetation at the foot of hills. Plains often flooded but heavily cultivated right up to river/stream bank in dry season. Monkeys seen on hills
Mangar	Cultivation limited due to rocky terrain and vegetation cover is substantial. Shrub land cleared in some areas for farming. A few vegetable farms present on land adjacent to river banks. Wood cutting and gathering common. Monkeys were seen on nearby hills and a monitor lizard was encountered close to a stream
Tambes	The terrain is very rocky but plains are heavily cultivated. There are strips of riverine vegetation which appear to be remnants of previously more extensive riverine woodland. No wild animals were seen.

### 2.3.1.1 Trapping of tsetse

Unbaited blue biconical traps (Laveissiere, 1973, Challier *et al.*, 1977 in FAO (1979), were deployed in each village for three consecutive days each sampling period. Each trap was supported on a metal pole such that the trap entrance was at a height of about 45 cm above the ground. Where necessary, vegetation was cleared for a radius of 3 m around traps to ensure and standardize trap visibility. The lower portions of the supporting pole were smeared with grease to prevent ants from climbing into the traps and destroying the catch. Traps were sited mainly along stream and riverine vegetation at intervals of between 100 and 150 m and included points along the river/stream such as bridges, cattle crossing or watering points, places where people washed and bathed. Figure 2.3 shows one of the traps in riverine vegetation.



**Figure 2.2: Biconical trap in riverine vegetation at one of the survey sites.**

In each village, some traps were also placed in the vicinity of households where cattle and other domestic livestock were corralled in order to trap any tsetse that might be present in such peri domestic settings as well as monitor the abundance of other biting flies. Traps were deployed from 0700 hours in the morning and subsequently checked every 24 hours for 72 hours to collect trapped flies (FAO, 1979). The number of traps varied between villages ranging from 11 to 27 depending on the size of the village and availability of suitable habitat (Table 2.3).

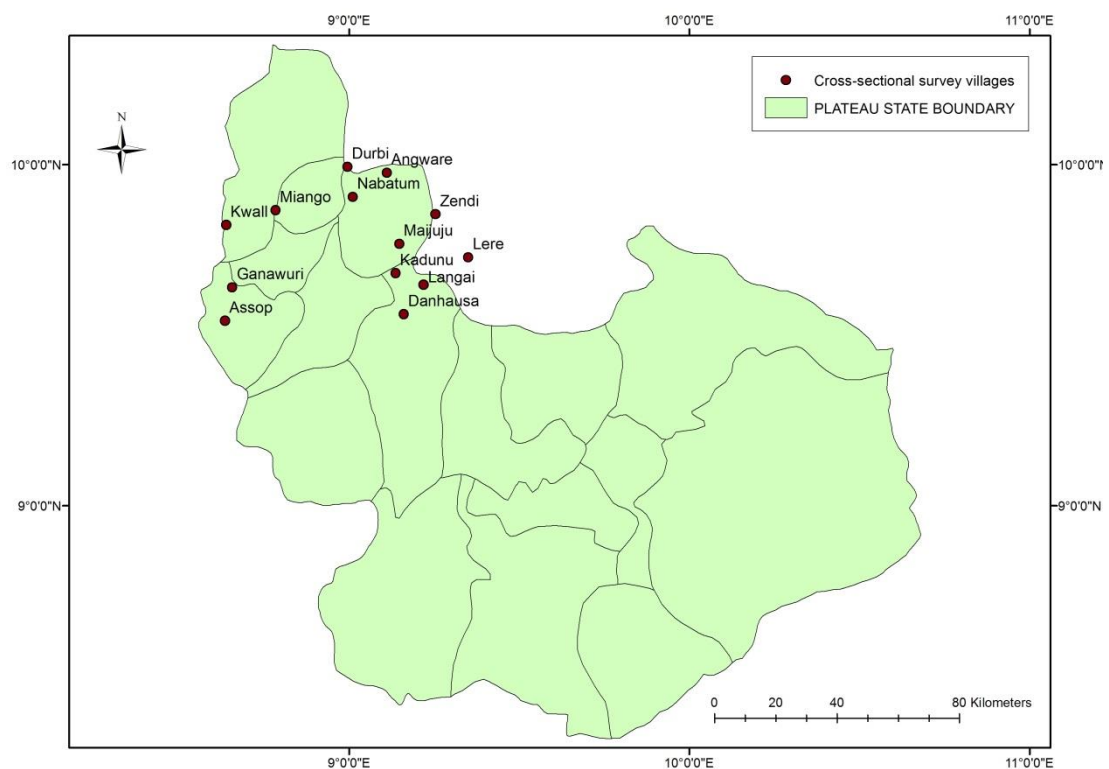
**Table 2.3: Number of Traps in Longitudinal Survey Villages**

<b>Village</b>	<b>Number of Traps</b>
Bokkos	11
Daffo	17
Hurti	27
Maiyanga	15
Mangar	17
Mbar	11
Tambes	17

The position of each trap was marked and geo-referenced using a hand-held Garmin Global Positioning System (GPS) Oregon450<sup>®</sup> (Garmin, USA) and remained unchanged throughout the survey.

### 2.3.2 Cross-sectional tsetse survey

Thirteen villages were selected for a cross-sectional tsetse survey to reflect a range of altitudes and included some villages that had previously been reported to harbour tsetse flies. This survey was designed mainly to investigate the effect of altitude on the presence and density of tsetse as well as determine if tsetse persisted in villages where they had been previously reported. It is thought that when combined with the results of the longitudinal survey, the results obtained from this survey would provide a clearer picture of tsetse distribution across a larger area of the plateau. The villages sampled were Nabatum, Durbi, Zendi and Maijuju and Angware in Jos East LGA; Assop and Ganawuri in Riyom LGA, Miango and Kwall in Bassa LGA, Langai, Kadunu, Danhausa in Mangu LGA and Lere in neighbouring Bauchi State (Figure 2.3).



**Figure 2.3: Cross-sectional Tsetse Survey Villages.**

Tsetse flies have previously been recorded in Maijuju, Zendi, Kadunu, Miango, Ganawuri and Kwall. Zendi which is on the boundary between the plateau and Bauchi State, and Lere in Bauchi were included because there is a constant movement of cattle from the plateau to these areas to exploit available grazing (Charles Dongkum, *personal communication*).

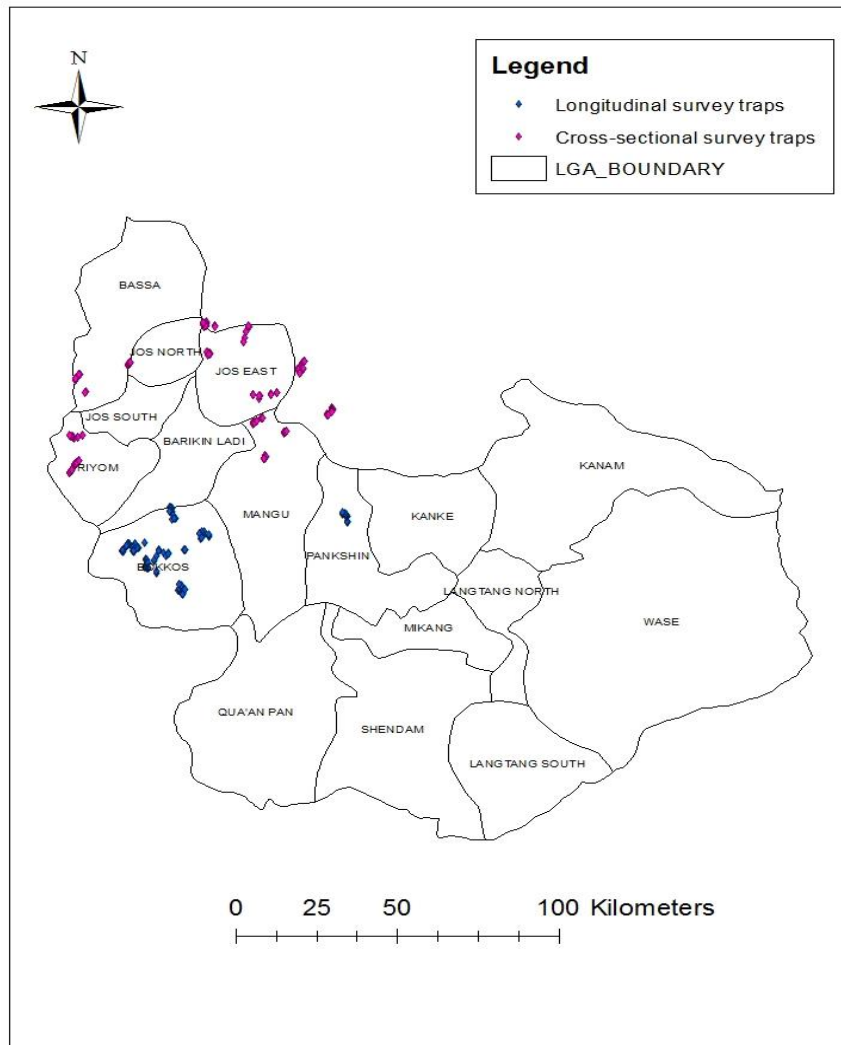
The land-use pattern in most of the villages is similar and includes a mix of cultivation, livestock production and some areas of natural vegetation along drainage lines. However, in Angware, Ganawuri, Miango, Kwall, Lere, Durbi, Maijuju, Danhausa, and Kadunu, cultivation of crops such as maize and tomatoes, along the banks of streams and rivers is a common practice whereas in Assop, Langai, Nabatum and Zendi, this practice is not commonly observed. Cattle are present in all villages.

Trapping of tsetse took place during the rainy season between July and August 2013 and procedures were similar to those described for the longitudinal survey except that in this survey, trapping was conducted only once for 72-hours in each village. Table 2.4 shows the altitude and number of traps for each village. Number of traps used in each village depended on availability of suitable trapping sites.

**Table 2.4: Altitude and Number of Traps in Cross-sectional Survey Villages**

Village	Mean Altitude (m)	Number of Traps
Miango	1145	10
Kwall	840	10
Ganawuri	1043	10
Assop	881	21
Nabatum	1337	10
Angware	936	11
Durbi	907	18
Maijuju	886	14
Zendi	786	20
Kadunu	1061	14
Danhausa	1076	10
Langai	988	10
Lere	842	13

Each trap location was geo-referenced as in the longitudinal survey and both sets of trap coordinates were imported into a GIS ArcGIS 10.2 and overlaid on a map of Plateau State (Figure 2.4).



**Figure 2.4: Trap locations for Longitudinal and Cross-sectional Surveys**

### **2.3.2.1 Identification preservation and transport of trapped flies**

The species and sex of captured flies was determined according to procedures and keys provided in the guidelines for the collection of entomological baseline data for tsetse area-wide integrated pest management programmes (FAO, 2008). Each whole fly was preserved separately in small plastic tubes containing 99.8% acetone. The tubes in which the flies were preserved were tightly sealed and labelled in pencil



indicating the species, sex, location, and date of collection. Samples were shipped under import licences PATH 176/2012/7 and PO/2013/03 to the Infectious Disease Laboratories, Division of Pathway Medicine of the University of Edinburgh and stored at -80° C for future molecular investigations.

#### **2.3.2.2 Collection of climate and vegetation data**

In order to relate tsetse presence/distribution to climatic variables, climate data (annual mean temperature, annual precipitation, minimum and maximum temperature) and altitude was obtained from WorldClim ([www.worldclim.org](http://www.worldclim.org)) which comprises a set of global climate layers; variables included in the worldclim dataset are monthly total precipitation, monthly mean, minimum and maximum temperature, and 19 derived bioclimatic variables. The data layers were generated through interpolation of average monthly climate data for the years 1950-2000 obtained from weather stations on a 30 arc-second resolution grid (often referred to as "1 km<sup>2</sup>" resolution). They were produced using data compiled by major climate databases including the Global Historical Climatology Network (GHCN), FAO, World Meteorological Organisation, the International Centre for Tropical Agriculture (CIAT), R-HYdronet and several other minor databases. Full details of the methods used to generate the data are described in Hijmans *et al.* (2005). The data was available as raster files which were imported into a GIS ArcGIS® for desktop version 10.2 to enable the extraction of actual values for each climatic variable using the extract-to-point tool in the Arc toolbox. The datasets are freely available for academic and non-commercial use.

### 2.3.3 Data Analysis

The apparent density of tsetse which is a measure of relative abundance was calculated as the number of trapped tsetse divided by the number of traps multiplied by the number of sampling days and expressed as flies per trap per day (FAO, 2008). The climate data were summarized and used to categorize villages in order to identify trends common to the villages where tsetse occurred. This was done using k-means clustering with the package ‘cluster’ in R (version 3.1) statistical Software (R core Team 2013).

## 2.4 Results

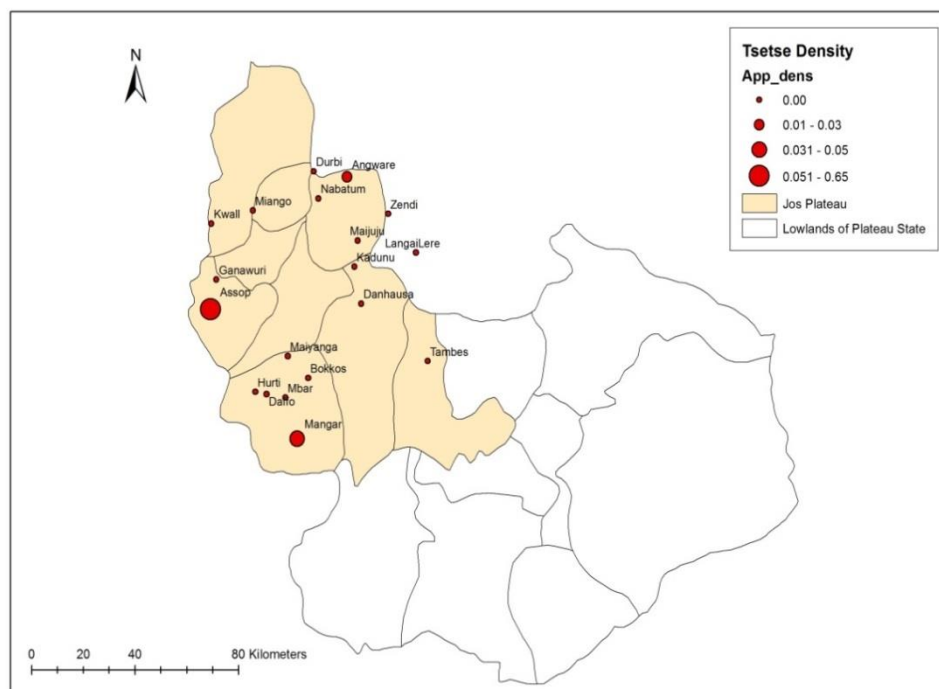
The results of both surveys are first presented separately and then combined to show overall distribution of tsetse across the sampled areas spread across the Jos Plateau.

### 2.4.1 Tsetse species and overall apparent density

In the thirteen three day trapping sessions conducted during the longitudinal survey from March 2012 to September 2013, 30 *G. p. palpalis* (17 females and 13 males) were trapped in Mangar in Bokokos LGA. In this village, tsetse flies were present on all streams where traps were deployed and the apparent density of tsetse over the whole survey period was 0.05 flies per trap per day (FTD). No tsetse flies were trapped in any of the other six villages included in this survey.

From the cross-sectional survey, 41 *G. p. palpalis* (21 females and 20 males) were trapped in Assop while one (1) male *G. tachinoides* was caught in Angware. Overall

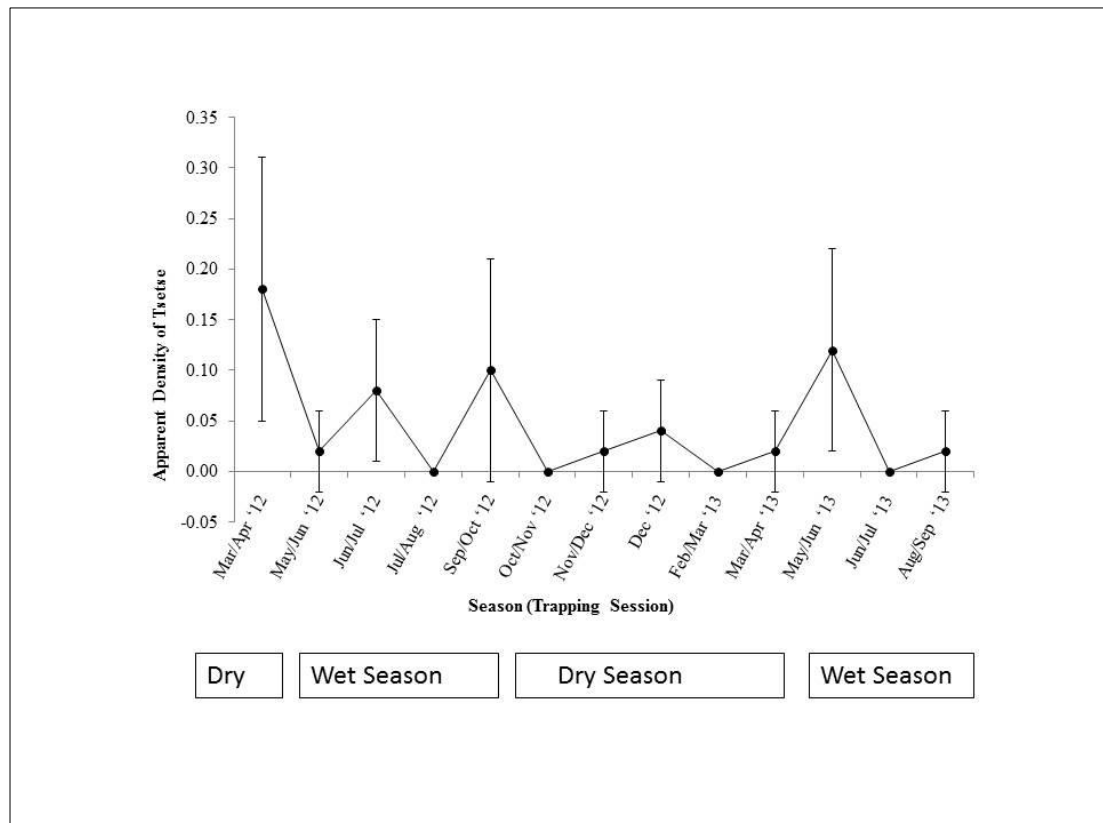
apparent tsetse density was 0.65 and 0.03 respectively in these two villages. No tsetse flies were trapped in any of the other 11 villages included in this particular survey. Thus from both surveys, tsetse flies were trapped in three of 20 villages; Assop and Mangar which lie close to the south and western edges of the plateau and Angware which is located on the south eastern margin of the Jos Plateau. These are presented in terms of apparent tsetse density in Figure 2.5.



**Figure 2.5: Apparent density of tsetse in the longitudinal and cross-sectional survey villages.**

### 2.4.2 Seasonal variation in tsetse trap catch in Mangar

Mangar was the only longitudinal survey village where tsetse flies (all *G. palpalis palpalis*) were caught and observed changes in the apparent density based on trap catches over the sampling period are shown in Figure 2.6.



**Figure 2.6: Mean monthly tsetse catch (Apparent Density) during longitudinal survey in Mangar.** Error bars indicate 95% confidence interval for Apparent tsetse density (mean number of tsetse/trap/day;  $n = 51$ ). Wet season (May-early October); Dry season: (November – March). A few scattered rain showers occur in March and April but the wet season proper, starts in May.

Generally, mean trap catch i.e. apparent density was low throughout the period and ranged from 0.00 to 0.18 flies per trap per day. There was no clear seasonal pattern in

the apparent density of tsetse although the highest single catch was recorded during the first sampling session in March/April 2012 at the end of the dry season.

### **2.4.3 Effect of climate altitude and vegetation on tsetse distribution and abundance**

The climatic variables for which records were obtained from WorldClim are summarized in Table 2.5.

**Table 2.5: Summary of Climatic Variables Means (SE) in Villages sampled for Tsetse flies on the Jos Plateau.**

Village	Annual Mean Temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Annual precipitation (mm)	Altitude (m)
<b>Angware</b>	<b>23.9 (0.12)</b>	<b>33.7 (0.14)</b>	<b>14.2 (0.1)</b>	<b>1231.0 (6.97)</b>	<b>936.44(21.94)</b>
<b>Assop</b>	<b>23.8 (0.56)</b>	<b>32.9 (0.64)</b>	<b>14.7 (0.4)</b>	<b>1453.7 (6.34)</b>	<b>881.66(106.13)</b>
Bokkos	21.5 (0.05)	30.4 (0.06)	12.6 (0.1)	1316.5 (4.8)	1256.26(13.06)
Daffo	21.6 (0.1)	30.5 (0.1)	12.9 (0.1)	1382.3 (4.19)	1270(29.68)
Danhausa	23.0 (0.04)	32.3 (0.07)	13.7 (0.1)	1252.3 (1.04)	1072.14(5.08)
Durbi	24.1 (0.08)	33.8 (0.11)	14.4 (0.1)	1258.6 (3.63)	905.77(20.42)
Ganawuri	23.6 (0.07)	32.7 (0.12)	14.6 (0.1)	1438.6 (3.69)	958.12(25.26)
Hurti	21.5 (0.11)	30.4 (0.11)	12.9 (0.1)	1396.2 (4.3)	1300.54(20.04)
Kadunu	23.2 (0.08)	32.5 (0.09)	13.9 (0.1)	1256.4 (4.88)	1058.5(16.55)
Kwall	23.9 (0.45)	33.2 (0.55)	14.7 (0.3)	1394.5 (5.05)	863.17(110.55)
Langai	23.4 (0.05)	32.9 (0.1)	13.9 (0.1)	1231.2 (1.55)	988.71(2.73)
Lere	24.3 (0.12)	34 (0.16)	14.3 (0.01)	1188.0 (3.06)	840.16(28.61)
Maijuju	23.7 (0.67)	33.2 (0.8)	14.1 (0.5)	1242.9 (8.77)	936.68(113.69)
Maiyanga	21.5 (0.38)	30.4 (0.4)	12.7 (0.3)	1346.7 (1.99)	1307.06(74)
<b>Mangar</b>	<b>22.7 (0.34)</b>	<b>31.7 (0.37)</b>	<b>13.8 (0.3)</b>	<b>1368.2 (3.42)</b>	<b>1052.92(58.95)</b>
Mbar	21.5 (0.45)	30.4 (0.48)	12.7 (0.4)	1355.1(12.35)	1284.2(51.88)
Miango	22.8 (0.05)	31.8 (0.05)	13.9 (0.01)	1361.3(0.95)	1170.37(20.93)
Nabatum	22.1 (0.01)	31.3 (0.08)	13 (0.1)	1298.8 (23.4)	1337.44(10.97)
Tambes	22.1 (0.01)	31.4 (0.05)	12.6 (0.1)	1168.0 (2.83)	1162.77(11.56)
Zendi	22.1 (0.01)	34.4 (0.26)	14.4 (0.1)	1196.1(4.7)	777.47(49.94)

Figures in brackets are Standard Errors of the Means of each variable. Rows highlighted in bold indicate villages where tsetse flies were found.

Cluster analysis was carried out using the package ‘cluster’ K- means clustering to characterize the villages based on the climatic variables in Table 2.5. Altitude, mean annual temperature and annual precipitation were the best predictors and separated the villages into four clusters. All three villages where tsetse flies were present clustered separately alongside those in which no tsetse flies were trapped. Only one cluster did not contain any tsetse infested village. This cluster contained villages with the highest altitude, lowest mean annual temperature and relatively high rainfall as shown in Table 2.6.

**Table 2.6: Clustering of villages based on selected climatic variables**

Clusters	Altitude (m)	Mean Annual temperature	Annual precipitation (mm)	Villages within cluster
1	873.44	23.54	1223.91	<b>Angware</b> , Durbi, Lere, Maijuju, Zendi
2	895.02	23.79	1434.60	<b>Assop</b> , Ganawuri, Kwall
3	1279.76	21.68	1359.68	Bokkos, Daffo, Hurti, Maiyanga, Mbar, Miango, Nabatum
4	1072.26	22.82	1260.92	Danhausu, Kadunu, Langai_gindiri, <b>Mangar</b> , Tambes

Villages where tsetse flies were found are highlighted in bold. Cluster 1= Low altitude, low precipitation, high temperature; Cluster 2 = Low altitude, high precipitation, high temperature; Cluster 3 = High altitude, high precipitation, high temperature; Cluster 4 = Medium altitude, low precipitation, medium temperature

## 2.5 Discussion

Recent studies that AAT which was historically rare and of little significance on the Jos Plateau, is now widespread with a prevalence of 46.8% (Majekodunmi *et al.*, 2013). Expansion in the distribution of tsetse vectors on the plateau is one of the

main reasons suggested for this change (Dede *et al.*, 2005; Majekodunmi *et al.*, 2013). However, previous studies on the distribution and abundance of tsetse on the plateau have been limited to a few villages representing only a small part of the plateau and have not demonstrated such an expansion. In this study, the current distribution and abundance of tsetse in 20 villages on the Jos Plateau were investigated using longitudinal and cross-sectional tsetse trap surveys.

### **2.5.1 Tsetse species encountered**

Two species of riverine tsetse flies of the subgenus *Nemorhina* (*G. p. palpalis* and *G. tachinoides*) were recorded in this study. *G. palpalis* occurred in two villages and was the predominant of the two species considering that only one *G. tachinoides* was caught in one, of the combined total of 20 villages sampled. The results are in agreement with those from previous tsetse surveys on the Jos Plateau (Abbah *et al.*, 2011; Dede *et al.*, 2005; Kalu, 1996a; Majekodunmi, 2006) which indicate that *G. palpalis* is the more common of the two species.

The presence of these two riverine tsetse species on the plateau can be attributed to the fact that riverine vegetation is the main tsetse habitat available in the area. Additionally, they are among the tsetse species known to feed rather indiscriminately; utilizing the most available hosts in their immediate habitat such as reptiles and birds as well as man, and his domestic animals (Weitz, 1963) which frequent these sites. As such, they are able to adapt to the disappearance of their wild hosts and survive in human modified habitats such as occur on the plateau. In this,



they are unlike tsetse of the *morsitans* and *fusca* groups that obtain blood meals mainly from wild vertebrate hosts (Weitz, 1963) which are rare on the plateau.

Species of the *morsitans* and *fusca* groups of tsetse have never been known to occur naturally on the Jos Plateau which does not have the savannah and forest habitats they respectively inhabit or the wild vertebrate hosts on which they are mostly dependent for blood meals. However, Onah (1995) trapped 25 *G. m. submorsitans* alongside *G. tachinoides* and *G. palpalis* in a cattle market in Jos, the capital of Plateau State where there was no natural vegetation or wild hosts. This is the only known record of this species on the plateau and it was suggested that the flies were accidentally brought into the market on trucks transporting cattle from areas of the country where *G. m. submorsitans* occurred naturally. The imported population was sustained by an abundant supply of blood meals from the cattle in the market while the market stalls provided shaded resting sites. It is hard to say whether the Jos Plateau may have harboured small populations of *morsitans* group tsetse in the past although as its vegetation was originally characterised by open woodland (Keay, 1953) this may have been possible

Currently, the plateau is dominated by open grassland except for remnants of woodland and strips of forest along rivers and streams and on the steep and less accessible margins on its south-western and eastern escarpments. However, it was observed even during this study that what is left of the riverine vegetation is being cleared to make way for vegetable plots sited on stream and river banks in several of

the villages surveyed. Such practices are likely to affect the long-term survival of the two riverine tsetse species currently found on the plateau.

### **2.5.2 Relative abundance of tsetse**

It is evident from previous studies (Kalu, 1996a; Dede *et al.*, 2005 and Majekodunmi 2006) (where apparent densities of tsetse ranged from 0.2 - 4.3 flies per trap per day), that tsetse flies are not abundant on the plateau although there may be wide variations in abundance perhaps due to the fragmented and patchy distribution of riverine habitats in the area. This trend was maintained in the current study evidenced by the absence of tsetse in 17 of the 20 villages surveyed, and the low apparent tsetse density recorded for the three villages where tsetse occurred: 0.05 flies per trap per day in Mangar, 0.03 flies per trap per day in Angware and 0.65 flies per trap per day in Assop. This perennially low density of tsetse on the plateau is probably an indication that the environment is not very suitable for tsetse. For instance, the generally low mean temperature of the plateau is likely to lengthen the duration of the puparial period which is temperature dependent (Glasgow, 1963; Nash, 1948). This invariably affects the reproductive rate of the flies.

#### **2.5.2.1 Seasonal variation in apparent density of tsetse**

As earlier mentioned, previous studies on the plateau, do not provide information on seasonal variations in tsetse density. In the present study, seasonal (longitudinal) data on tsetse abundance was obtained only in Mangar as no tsetse flies were found in the other longitudinal survey villages. However, due to the small number of tsetse

trapped (30) over the 18-month period, it was difficult to draw any conclusions on this subject. The highest apparent density recorded was at the end of the hot dry season in March/April 2012. The higher density recorded during this period is likely to have been a result of fly concentration in shaded parts of streams such as was observed in Katabu also in north central Nigeria by Nash & Page (1953). No flies were caught in the wettest months (July and August) of both years of the current study and this was unexpected as tsetse are supposed to be abundant during this period. However, since trap catches are dependent not only on the abundance of tsetse but also on their flight activity, the heavy rain showers which occurred during the trapping days in July could have made the flies inactive and kept them from being caught in the traps. It has also been suggested that the peak of the rains is also the period of maximum dispersal of tsetse along riverine vegetation and sometimes outside into the surrounding savannah (Nash, 1948; Nash & Page, 1953) and it is possible that the flies in Mangar also became more dispersed along the streams during this period. Further studies could be conducted to determine more precisely, the seasonal variations in fly density in this area.

### **2.5.3 Habitats occupied by tsetse**

The preferred habitat of tsetse in the subgenus *Nemorrhina* or *palpalis* group is riverine vegetation, which is why they are referred to as riverine tsetse. However, they have been known to occupy atypical, peri-domestic or man-made habitats such as orchards or plantations as long as these provide suitable shade and humidity. Early observations of such peri-domestic behaviour in *G. tachinoides* were made in southern Nigeria (Baldry, 1964) and more recently, Ahmed (2004) also reported the

presence of a stable, breeding population of *G. palpalis* in Kontagora, a densely populated town in Niger State to the west of the plateau. In the current study, tsetse flies were strictly restricted to riverine vegetation and no flies were caught in peri-domestic habitats in the vicinity of cattle corrals and other animal enclosures where but many houseflies, *Stomoxys* and a few Tabanidae were caught in such locations (see Chapter 4).

The absence of these two shade-loving species of tsetse in these sites may be due in part, to the lack of shade in the form of dense bush or thicket in the areas around human dwellings in the surveyed villages as most of these were occupied by cultivated farmland or open grassland. A detailed study by Nash and Page (1953) on the ecology of *G. palpalis* in Katabu about 130 km northwest of Jos, demonstrated how important riparian tree cover was for the survival of this species especially during the harsh months of the dry season. The high canopy of the riparian vegetation provided vertical insulation from the sun while an undergrowth of creepers and shrubs kept out hot dry winds from the surrounding savannah.

Peri-domestic behaviour has been observed in *G. tachinoides* and was strongly associated with its preference for feeding on domestic pigs kept in villages (Baldry, 1964, 1969; Dede *et al.*, 2005; Kuzoe *et al.*, 1985). The absence of this tsetse in peri-domestic sites tsetse in the areas currently surveyed could be due to the absence of domestic pigs in most of the sites sampled as Fulani do not keep pigs because of their Islamic beliefs. The observation that the riverine habitats where tsetse occurred are

frequented by cattle and people, suggest that the flies are guaranteed a steady source of blood meals and do not need to adopt a peri-domestic orientation in order to secure a consistent food supply.

In most other studies of tsetse on the plateau (Abbah *et al.*, 2011; Kalu, 1991, 1996a, 1996b; Majekodunmi, 2006), all the tsetse flies trapped were caught from stream/riverine vegetation except for Dede *et al* (2005) who reported the presence of *G. tachinoides* in peri-domestic habitats in Federe while Onah (1995) also detected tsetse living and breeding in a cattle market in Jos town. There might be a need to re-visit the areas surveyed by Dede *et al.* (2005) to determine if *G. tachinoides* still occurs in peri-domestic sites.

#### **2.5.4 Spatial distribution of tsetse**

Recent work by Majekodunmi *et al.* (2013) showed that bovine trypanosomiasis has a wide spatial spread on the plateau and the implication was that the tsetse vectors responsible for transmission were also widely distributed. However, the spatial distribution of tsetse as indicated by the presence of tsetse only in Assop and Mangar close to the south-western edge and Angware on the south-eastern edge of the plateau does not support such a view. The results from this study show that tsetse flies are confined to marginal areas of the plateau rather than being widely distributed. It appears that in such areas, the rocky and often steep terrain favours tsetse by limiting human settlement and cultivation thus ensuring the availability of

dense, natural vegetation on the banks of streams and rivers that flow off the plateau in these areas.

This reasoning is supported by observations made by Nash (1948) during an epidemic of sleeping sickness among the people who lived in Rukuba on the western escarpment of the plateau at an altitude of 3200 feet (~975m). According to his description, the area between Jos (in the centre of the plateau) and Rukuba consisted largely of treeless grassland and rocky hills and many streams flowed westward towards the escarpment. However, because their banks were devoid of vegetation, the streams and the surrounding grassland were tsetse-free. At the edge of the escarpment, the streams descended about 400 feet, down rocky gorges choked with evergreen vegetation which supported large numbers of *G. palpalis*. On the plains below the escarpment, the stream banks were once more treeless and free of tsetse.

This account by Nash is one of the earliest records of tsetse on the Jos Plateau and shows that *G. p. palpalis* was confined to the edges of the escarpment due to the presence of dense riverine vegetation supported by the fast flowing rocky streams in these areas; a situation which was observed in Assop and Mangar. Both of these villages lie close to the south west escarpment of the plateau and are not as heavily populated or intensively farmed as the other villages surveyed because of their rocky terrain. Assop is supplied by many streams which combine and flow down steep cliffs into the River Kaduna to the northwest of the Jos Plateau. Mangar also has

streams and rivers which are tributaries to two main rivers: Dep and Mada which drain into the River Benue in the plains at the southern edge of the plateau.

Angware, the only village where *G. tachinoides* was detected, lies in a valley on the north-eastern margin of the plateau and a big river, 'Kogin Jarawa' (River Jarawa in the Hausa Language) runs through this village and drains into rivers that flow north-east to Lake Chad. According to some of the inhabitants of the village spoken to during the survey, the vegetation on this river was once heavily infested by tsetse flies which have declined and almost disappeared due to extreme fragmentation of the riverine vegetation. This fragmentation is due to a significant increase in agricultural and other human activities since the village was made the headquarters of Jos East LGA. It was also observed that the river banks and adjacent land were lined with tomato plots and maize farms in place of natural riverine vegetation.

Furthermore, of the seven villages where tsetse had previously been reported (Kadunu, Kwall, Ganawuri, Miango, Maijuju, Zendi and Assop, it was only in Assop that tsetse were found in the current study. This particular finding suggests that tsetse flies are neither becoming more abundant nor is their distribution expanding on the plateau. On the contrary, it is more likely that over the years, they have declined due to the destruction of their habitats. It is apparent from this study that tsetse flies are present only towards the edges of the plateau where the steep hilly terrain ensures the survival of some of their natural riverine habitats. The central parts of the plateau which are densely populated and heavily cultivated are likely to be tsetse-free.

### **2.5.5 The effect of climate and altitude on tsetse distribution**

Not much variation was observed among the climatic variables investigated between the villages surveyed for tsetse. However, it was noted that tsetse were found only in villages where the maximum altitude did not exceed 1100 m. Apart from the lower mean temperatures in the high altitude areas, they are also more densely populated and heavily cultivated. This limits availability of tsetse habitat to lower areas on the escarpments with sparse human populations and more riverine vegetation. Clearly, other climatic variables such as temperature and rainfall are likely to interact with altitude in influencing tsetse presence.

Based on the clustering of villages by annual mean temperature, altitude and annual rainfall, it is apparent that many of the villages shared characteristics which would favour the survival of tsetse. However, there may have been a missing component which could not be identified by the minimal analysis conducted in this study. It is recommended that future studies attempt to model tsetse distribution on the plateau using more climatic variables obtainable from remotely sensed data as described by Rogers *et al.* (1996) and Rogers (2000). In combination with the data collected from the present study and previous studies on the plateau, future studies could more accurately describe habitats suitable for tsetse on the plateau.

Even though no statistically significant relationship was found between the selected variables and tsetse presence (see Table 2.6), observations made during the surveys indicate that the presence of dense vegetation on the banks of streams and rivers is a



crucial factor determining the presence of tsetse. Tsetse flies were present only in villages where the riverine vegetation was denser and less fragmented. Even in such villages, flies were restricted only to sections of riverine vegetation that were not much affected by cultivation. Future work could examine more closely the effect of riverine vegetation and the extent of habitat fragmentation on tsetse presence on the Jos Plateau.

### **2.5.6 Conclusion**

The results of the surveys conducted in 20 villages on the Jos Plateau to determine the distribution and density of tsetse indicate that *Glossina palpalis* and *G. tachinoides* are present in a few villages with *G. palpalis* being the more abundant species. Both species were found only in riverine habitats and did not occur in peri-domestic habitats perhaps due to the absence of suitable shaded peri-domestic habitats and the sufficiency of hosts in their riverine habitats. The distribution of the flies across the plateau is very restricted contrary to suggestions that tsetse fly populations have expanded.

Indeed, results obtained in this study indicate that tsetse might be declining. It is proposed that the most likely reason for this decline is the destruction of the riverine habitats favoured by the species of tsetse present on the plateau. It was observed that the rivers and streams where tsetse flies were caught are frequently used by villagers for washing, bathing and watering cattle. Cattle are also grazed on grasses along stream banks which have not been taken over by vegetable plots. Consequently, even

though tsetse density as determined by trap catches are low, there would still be opportunities for transmission of trypanosomes between tsetse and cattle which may partly explain the rise in AAT prevalence on the plateau. However, as tsetse flies do not occur widely across the plateau, there are likely to be other factors behind the wide spatial distribution of bovine trypanosomiasis recently reported in the area and these need to be identified.

### **3 Seasonal Migration and Animal Trypanosomosis in cattle on the Jos Plateau**

### 3.1 Introduction

The problem of African animal trypanosomosis (AAT) on the Jos Plateau in north central Nigeria became apparent around the early 1980s (Joshua, 1982). Its emergence is often attributed to the invasion of the plateau around the same period by tsetse flies. But as pointed out earlier in section 1.2.6 of Chapter 1, *G. p. palpalis* at least, was present on the south western escarpment of the Jos Plateau long before the 1980s (Nash, 1948; Taylor, 1930) and so the claim that AAT emerged on the plateau following invasion by tsetse is incorrect. A more likely explanation is that as many more Fulani cattle-keeping pastoralists immigrated from the far north and surrounding areas to settle on the plateau, it led to a sharp increase in cattle numbers such that contact between tsetse and cattle became more frequent resulting in higher incidence of AAT.

A recent survey of 7142 cattle sampled from 30 villages representing wide spatial coverage of the Jos Plateau found the overall AAT prevalence to be 46.8% (95% CI: 39.0% - 54.5%) and in some villages, prevalence was close to 100% (Majekodunmi *et al.*, 2013). The trypanosome species involved were *T. congolense*, *T. vivax* and *T. brucei* and accounted for 27.7% (21.8 - 33.6%), 26.7% (18.2 - 35.3%) and 3.2% (2.8 – 3.6%) respectively of the detected infections. These results were puzzling as most of the plateau is free of tsetse, and so there is no clear explanation for the wide spatial distribution and high prevalence of infections detected in this study. Previous studies (Dede *et al.*, 2005; Majekodunmi, 2006) have shown that *G. p. palpalis* and to a lesser extent *G. tachinoides*, are present in a few areas (usually at the edges) of the Jos Plateau at low apparent densities. Similar results were obtained from the

surveys outlined in Chapter two of this thesis; *G. palpalis*, which has been shown to be a competent vector of *T. vivax* strains from Nigeria (Moloo *et al.*, 1987) but a poor vector of *T. congolense* (Moloo & Kutuza, 1988) was present in only two of 23 surveyed villages and a single *G. tachinoides* was trapped in a third village.

Due to the relative absence of tsetse, mechanical transmission by other biting flies such as Tabanidae and *Stomoxys* has been suggested as an alternative means by which cattle on the plateau become infected with trypanosomes. However, even though some species of these biting flies have been collected from the area (Abba *et al.*, 2011; Dede *et al.*, 2005), there is no clear evidence that they are involved in transmission of the disease. An attempt is made to explore this possibility in another chapter (Chapter 5) of this thesis.

A more likely reason suggested by previous workers (Anosike *et al.*, 2003; Kalu, 1995, 1996; Kalejaiye & Omotainse, 2000; Kalejaiye, 2006; Majekodunmi *et al.*, 2013; Qadeer *et al.*, 2008, Yanan *et al.*, 2007) as contributing to the widespread occurrence of AAT on the plateau, is the practice of transhumance or seasonal migration of Fulani cattle in search of pasture and water during the dry season. It is speculated that such cattle become infected during migration to lower altitude areas surrounding the plateau; where it is also presumed that tsetse are present at higher densities. This theory though only minimally investigated on the plateau, is not without merit as between the 1920s and 1960s, AAT in northern Nigeria was closely linked with the annual migration of Fulani cattle from the largely tsetse-free semi-

arid north to areas in the south (where denser tsetse infestations occurred) in search of grazing and water during the dry season (Glover, 1965). Cattle were also trekked to markets in the south along the same routes and exposed to the risk posed by tsetse (Godfrey *et al.*, 1965). Tsetse flies, in particular, *G. m. submorsitans* with high trypanosome infection rates (up to 68%) occurred on these routes and fed vigorously on the herds that provided an easy and abundant source of blood meals (Jordan, 1965; Riordan, 1977). Due to the pathogenic effects of the trypanosome infections and the added strain of the usually long trek, some of the animals died or were slaughtered for sale, while those which survived returned to their home villages in the north at the start of the rainy season (Glover, 1965). Such survivors were likely to harbour trypanosomes which could have been transmitted to tsetse and other cattle when they returned.

Recent observations have been made, of changes in the patterns of Fulani cattle migration on the Jos Plateau and the trend is one of increasing frequency and duration (Majekodunmi *et al.*, 2014). However, no studies have been specifically directed at determining the contribution of these increased migratory movements to the prevalence of AAT in the area; perhaps because most of the Fulani in this area are settled agro-pastoralists and are not known to move their animals far off the plateau during the seasonal migration (Awogbade, 1979, 1983). It is within the context of these observations and a project aimed at understanding the spread of AAT on the plateau with a view to reducing its impacts that the present study exploring the link between AAT and seasonal transhumance was conducted. Before

the details of the study are presented, a brief description of Fulani agro-pastoralism and patterns of seasonal migration specific to the Jos Plateau along with factors responsible for the observed changes in these patterns are presented to provide additional background.

### **3.2 Fulani Agro-Pastoralism on the Jos Plateau**

Fulani pastoralists and their cattle are believed to have entered and settled on the Jos Plateau sometime between 1906 and 1910; attracted by the cool climate, relative absence of tsetse fly vectors of AAT and at the time, low human population and the almost year-round availability of abundant pasture (Awogbade, 1983). These factors particularly suited the extensive production system practiced by the Fulani in which cattle are freely grazed on untended natural pastures requiring little input in terms of supplementary feeds. (Awogbade, 1983; Blench, 1994).

Since the initial immigration event in the early 20<sup>th</sup> century, many more pastoralist Fulani have settled in the area (Blench, 1994) and it is estimated that around 300,000 pastoral Fulani currently live in different parts of the Jos Plateau and that they own around 1.05 million heads of cattle. This represents approximately 95% of the cattle population in the area and ~7% of the national herd (RIM, 1992). Most Fulani settlers on the plateau have also taken up small scale crop farming and are thus described as agro-pastoralists although for most, livestock husbandry remains the main source of livelihood (Awogbade, 1983; Majekodunmi *et al.*, 2014).

### **3.2.1 Composition and size of herds**

The majority of the cattle kept by Fulani in this area are trypano-susceptible indigenous *Bos indicus* genotype commonly called zebu of which the Rahaji or white Fulani is most common (Awogbade 1983; Majekodunmi *et al.*, 2014). It is favoured for its high milk yields (White and Wickens, 1976), good beef production, and adaptability to a variety of environmental conditions; enabling it to thrive in both high and low altitude areas of Cameroon and Nigeria (Felius, 1995 in ILRI, 2007). Some Fulani also keep a small number of Bunaji brown cattle and white Fulani x Bunaji brown crosses, which are primarily used to improve meat yields (Majekodunmi *et al.*, 2014). Herd sizes in individual households range from seven to 5015 cattle with an average of 253 or as low as 78 per household when a few outliers of over 1000 animals were excluded (Majekodunmi *et al.*, 2014). Herds are dominated by cows kept for milk and breeding purposes. Many Fulani households also keep a few sheep, goats and chicken, but cattle are the main and preferred livestock (Majekodunmi *et al.*, 2014).

### **3.2.2 Interactions with indigenous host communities**

Following their arrival, the Fulani settled and lived in relative peace among the indigenous host farming communities, which mainly grew cereals such as millet, acha/fonio, and corn (Hickey, 1978; Blench, 2004). Mutually beneficial relationships developed between the settlers and their hosts and included the exchange of milk, cheese and meat produced by Fulani cattle for grains grown by the farmers. Also, during the early part of the dry season (October-December) when natural pastures became depleted, Fulani cattle were allowed to graze on crop residues on the farms



and in return, the dung produced by the animals served to fertilize the fields on which they grazed (Hickey, 1978; Awogbade, 1983). In the latter part of the dry season when crop residues were exhausted and streams had dried up, the animals would be moved away from the villages to nearby lowlands or riverine valleys and return to the village at the start of the rains in what is described as seasonal transhumance.

Previously, Fulani did not purchase the land on which they settled, and were usually allocated land by the village head or local chief. They built homes on such land and grazed their animals on available fallow land (Hickey, 1978). However, in a recent study, Majekodunmi *et al.* (2014) reported that of 66 studied Fulani households on the plateau, all of them owned some land; with 61% owning between one and eight hectares. Most of the land owned by each household was used to grow crops while a small part of the land was used to tether or corral animals at night but animals were grazed extensively on communal fallow land (Majekodunmi *et al.*, 2014).

#### **3.2.2.1 Conflict between Fulani pastoralists and indigenous farmers and its effect on seasonal migration**

Conflicts between Fulani herders and indigenous crop farmers on the plateau and elsewhere, especially resulting from accidental damage to crops by cattle while grazing were not uncommon in the past (Awogbade, 1979; Blench, 1994). However, these were usually resolved effectively through traditional institutions with the affected farmer being compensated by the owner of the offending cattle (Blench, 1994; 2003; Majekodunmi *et al.*, 2014). In some villages on the plateau, conflict was

minimised by mutually agreed demarcation of the land into farms, grazing areas and access routes to these grazing locations (Blench, 2003). The beneficial exchange relations between pastoralists and farmers mentioned previously might also have acted as incentives for quick resolution of conflicts.

Over time, and due to rising human populations and the accompanying demand for more food crops, competition between farmers and pastoralists for access to land and water has become more intense. Fallow lands once freely available to the Fulani for grazing their cattle are now under cultivation and where some fallow land is still available, access routes to such land is blocked by farms (Blench, 2003). Changes in farming practices on both sides also seem to have affected the balance of exchange relations between Fulani and farmer. For example, many Fulani now grow their own grains and other food items; buying what cannot be grown from the market. Likewise, indigenes no longer depend exclusively on the Fulani for fresh milk and small sachets of powdered milk can easily be bought from local shops and markets (Blench, 2004; Majekodunmi *et al.*, 2014). Furthermore, the availability of cheap, petrol powered water pumps has also encouraged the expansion of irrigated vegetable production leading to a shift from the production of many of the cereals whose residues after harvest served as fodder for Fulani cattle during the early dry season. The vegetables which are often grown on riverine plots limit cattle access to watering points and do not provide beneficial residues after harvest for cattle to feed on (Blench, 2004).

These changes are seen as having contributed to rising tensions between the Fulani and indigenous farmers in many parts of the plateau and beyond. And as the traditional means of conflict resolution and avoidance appear to have broken down, frequent violent confrontations resulting in severe losses of human life, cattle and other forms of property has become a regular feature of the relationship between these two groups (Blench, 1994; 2004; Majekodunmi *et al.*, 2014). Consequently, there has been an increase in the frequency and extent of Fulani cattle movements. It is also no longer a matter of moving away in search of grass and water when they are not available, but one, of moving even when these resources are available in order to avoid conflicts which could result in violence if damage is done to crops during watering and grazing of cattle. In extreme cases, some Fulani have reportedly left the plateau and settled temporarily in the neighbouring States of Kaduna, Nasarawa and Bauchi (IRIN, 2009).

### **3.2.3 Seasonal transhumance**

The classic pattern of seasonal transhumance practiced by the Fulani on the Jos Plateau differs in some respects from the long distance, north-south, dry season movements practiced by Fulani in the more northerly semi-arid parts of Nigeria. Although it usually takes place in the dry season, it begins a bit later and lasts a shorter period than in the far north. Distances moved from permanent homesteads to dry season locations are also relatively shorter (Awogbade, 1979, 1983). This was largely due to the more amenable guinea savannah climate of the plateau and the fact that grazing and water is found in nearby valleys and during the latter part of the dry season by moving about 40km to areas in the surrounding plains (Awogbade, 1979).

Blench (1994) noted that in the more humid south of Nigeria, seasonal migration of Fulani can be complex and routes, distances moved, locations moved to and time spent varied widely depending on social and environmental circumstances and this probably applies also to the Jos Plateau.

Recently, Majekodunmi *et al.* (2014) observed that Fulani seasonal migration on the Jos Plateau which was formerly limited to the dry season (usually November to March/April), is now practiced during the wet season as well. They reported that 30 (46%) of 66 sampled households, undertook both wet and dry season migrations. The main reason for wet season migration was that majority of the fallow land that once served as wet season pastures could no longer be used as the cattle routes/tracks to these pastures had been blocked by farms. In many cases, these previously fallow lands had also been cultivated by the indigenes who claim original ownership of the land. As a result, cattle herds are moved to areas that are at lower altitudes, more sparsely populated and less cultivated within or outside Plateau State in order to access grazing and water. This is done also to avoid conflicts over crop damage by their cattle in the home villages; an event more likely to occur during the wet (main farming) season. These constant and sometimes extensive movements may increase opportunities for contact with disease vectors and agents including, but not limited to tsetse and trypanosomes.

### **3.3 Objectives**

Based on the foregoing, this study is aimed at identifying the role played by seasonal transhumance in the transmission and spread of AAT in cattle on the plateau. The specific objectives are:

1. To determine if tsetse flies are present along a cattle migration route used by cattle on the plateau.
2. To investigate the incidence of trypanosome infections in a herd of cattle over a period of migration encompassing both wet and dry seasons

### **3.4 Materials and Methods**

The steps taken to achieve these objectives involved the identification of a migrating herd, trapping of tsetse along its route and periodic collection of cattle blood samples which were screened for trypanosomes using molecular techniques. Details of these are described in the following subsections.

#### **3.4.1 Ethical statement**

All aspects of the study outlined below were approved by the Ethical Committee on Animal Use and Care at the Nigerian Institute for Trypanosomiasis Research (NITR) Vom, Nigeria and by the Department of Livestock Services, Plateau State Ministry of Agriculture. The cattle herd enrolled in the study was selected and sampled with the agreement, and under the supervision of the owner. Also due to issues of security, permission was sought from village heads before the commencement of tsetse

trapping in any village. They were informed of the purpose and duration of the survey and team members were properly introduced.

### **3.4.2 Selection of migrating cattle herd**

It was noted that different Fulani households trekked their cattle to different destinations via different routes (A. Majekodunmi, unpublished field data). Therefore, the intention was to select at least four herds migrating in two directions and to two separate destinations in order to detect any differences in the tsetse and trypanosomiasis risk associated with different routes and locations. Fulani herd owners in villages of Bokkos LGA where longitudinal tsetse surveys had been conducted (Chapter 2) were told of the study and its possible benefits and asked for their participation. However, only one person agreed to participate and his herd was eventually selected. It is not clear why others declined but they may have anticipated that it would require much time due to the longitudinal nature of the study. A few other herd owners that had earlier indicated interest were not sure if, when and where they would migrate. The pastoralist who agreed to participate was a student at the College of Animal Health in Vom near Jos the Plateau State capital, and the fact that he was knowledgeable about formal, animal health research may have encouraged his participation.

### **3.4.3 Tsetse sampling**

The selected herd migrated from Daffo to Fokolded and tsetse sampling was carried out in Maiduna Richa, Gyel Bali and Kadim on the route taken by the herd and at

Fokolded where the journey terminated. Sampling took place in each of the four villages between the 12<sup>th</sup> and 17<sup>th</sup> of July. Daffo, the home village, and Mangar along the route were excluded as they had already been sampled in a previous longitudinal tsetse survey described in Chapter 2. Traps were sited in suitable tsetse habitats (within riverine vegetation) and in areas where according to the herd boys, the herd grazed and watered during its migratory journey. Each trap location was georeferenced using a GPS Oregon450<sup>®</sup> (Garmin, USA). Traps were checked every 24 hours to collect trapped flies and were removed after three days. A second round of trapping was conducted in Gyel Bali at the same trap sites between the 28<sup>th</sup> and 31<sup>st</sup> of August. This was done to provide a larger sample of flies in anticipation of studies on trypanosome infection rates in tsetse to be presented in the next chapter as this village yielded a high number of flies in the earlier round of trapping compared to the other villages. It was of additional interest because it was the location where the herd under investigation spent the previous dry season from November 2012 to March, 2013. Deployment and checking of traps in Kadim and Fokolded were done on foot as the road linking these villages was steep and badly eroded due to much trampling by cattle which was made worse by the heavy rains in July.

#### **3.4.4 Identification, preservation and transport of tsetse samples**

Trapped flies were identified, preserved and transported as described in section 2.3.2.1 of chapter 2.

### **3.4.5 Sampling of cattle for trypanosome infections**

A portion of the herd was selected to monitor the incidence of trypanosome infections from June 2013 until the animals returned to Daffo. The herd was scheduled to return to Daffo in October but stayed much longer and sampling continued until March 2014. It was not possible to sample the herd before it departed Daffo in order to determine trypanosomiasis prevalence before migration due to a temporary loss of communication with the farmer and the earlier departure (planned for May but the rains were early). However, a month before departure, each animal had received a curative dose (7mg/kg of body weight) of diminazene aceturate and two weeks later, a prophylactic dose (0.5mg/kg) of isometamidium chloride. Both treatments were administered by the owner according to the dosage recommended by the manufacturers. It was therefore assumed that any trypanosome infections present at the commencement of migration would have been eliminated as there have been no reports of resistance to these drugs by trypanosomes on the plateau although this cannot be completely ruled out. Nevertheless, any trypanosome infections detected during the subsequent sampling times were considered as having occurred after migration from Daffo.

#### **3.4.5.1 Selection of cattle for blood sampling**

The number of animals included in the sample was 72 and this was in some way a convenience sample as it was the maximum number the owner allowed. This limit was set by the farmer in order to minimize the time that sample collection took off grazing, as well as the time and effort required to restrain the animals. To randomize the selection of the sample, all 120 animals in the herd were ear-tagged for easy



identification and the tag numbers were written on pieces of paper which were folded and placed in a bag. Seventy-two of these were drawn blindly from the bag to generate a random sample of 72 animals whose tag numbers corresponded to the selected pieces of paper. See Table 3.1 for the distribution of the sample according to age group and sex.

**Table 3.1: Sample Characteristics (age group and sex).**

Age group	Male	Female	Total
Juvenile (6months - 2years)	12	13	25
Adult (>2years)	19	28	47
<b>Total</b>	<b>31</b>	<b>41</b>	<b>72</b>

#### **3.4.5.2 Collection of blood samples**

Blood sampling was carried by trained veterinary staff from the National Institute for Trypanosomiasis Research, Vom. Each animal was restrained by the herd boys and sometimes by the owner who was present during each sampling exercise. Blood was collected from the jugular vein and spotted directly onto FTA™ cards (Whatman Bioscience Ltd. Cambridge, UK) (Figure 3.8). Each blood spot was labelled underneath with the ear tag number and colour as well as the age, sex and body condition score of the animal sampled. Samples were air dried overnight and stored in sealed waterproof pouches containing a silica gel desiccant (Sigma Aldrich, USA) in which they were shipped to the University of Edinburgh for molecular analysis.



**Figure 3.1: Blood sample collection and spotting onto FTA card.**

Sampling was carried out approximately every two weeks from June 6<sup>th</sup> 2013 to September 28<sup>th</sup> 2013 (wet season) and subsequently, once a month from October 28<sup>th</sup> 2013 to March 2014. The initial fortnightly sampling was to enable the detection of infections which may have been missed at a previous sampling point due to low or fluctuating parasitaemia and the pre-patent period before infections become detectable (Hoare, 1972) and also to closely track the occurrence of new infections. The frequency of sampling had to be reduced to once a month following complaints from the farmer that the fortnightly sampling was too cumbersome.

### **3.5 DNA extraction and elution from FTA cards**

To extract and elute DNA from the blood samples spotted onto FTA cards, five discs each of which had a diameter of 3 mm, were cut from different parts of each sample using a Harris punch and placed into a sterile 1.5 ml Eppendorf tube (Alpha laboratories, Eastleigh, UK). Five blank punches were made on clean filter paper between every sample to avoid cross contamination. One millilitre of Whatman FTA

purification reagent (Scientific Laboratory Supplies LTD, Nottingham, UK) was added to each tube containing the discs using a 5 ml graduated Pasteur pipette (Scientific Laboratory Supplies LTD, Nottingham, UK). The samples were gently agitated on a rocker for 15 minutes to facilitate the wash process aimed at removing haemoglobin from the samples. The liquid was carefully removed from each tube with a fine tip pastette ensuring that the discs were not accidentally removed and the wash was repeated one more time with FTA purification reagent. Two more 15-minute washes were done using 1 ml of 1.0 M Tris-HCl, pH 8, containing 0.1M Ethylene-diamine Tetra-acetic Acid (Tris-EDTA) buffer (Sigma-Aldrich Ltd. Gillingham, UK) to remove residual FTA purification reagent. After the wash steps, the discs from each tube were transferred to a PCR tube (Alpha laboratories, Eastleigh, UK) using a 200 µl pipette tip (Starlab Ltd., UK), and left to dry overnight at room temperature. DNA was eluted from the discs by adding 100 µl of 5% (w/v) chelex solution Chelex<sup>®</sup> 100 (Sigma Aldrich Ltd, UK) to the discs in each PCR tube and incubating at 90°C for 30 minutes in a Peltier thermal cycler. Extractions from blank filter paper punches were included to serve as negative controls during the subsequent PCRs.

### **3.5.1 Trypanosome detection in eluted DNA by ITS-1 PCR**

Trypanosome infections in the eluted DNA from the blood samples were detected by PCR using generic primers which amplify a segment of the internal transcribed spacer 1 (ITS-1) located between two highly conserved regions of ribosomal DNA (rDNA) (Njiru *et al.*, 2005). Variations in the length of the amplified fragment produced by different trypanosome species enable the simultaneous identification of

all known pathogenic African trypanosomes. See Table 3.2 for primer sequences and Table 3.3 for PCR product band sizes used to differentiate trypanosome species and sub-species.

**Table 3.2: ITS-1 PCR Primer sequences (Njiru *et al.*, 2005).**

PCR Target	Primer Name	Sequence (5'-3')
<i>Trypanosoma</i> spp. ITS1 rDNA	ITS1-CF ITS1-BR	CCGGAAGTTCACCGATATTG TTGCTGCGTTCTTCAACGAA

**Table 3.3: Expected band sizes for the pathogenic African trypanosomes following ITS-1 rDNA PCR (Njiru *et al.*, 2005).**

Sub-genus	Species/Sub-species	Expected band size (bp)
<i>Duttonella</i>	<i>T. vivax</i>	250
<i>Trypanozoon</i>	<i>T. b. brucei</i> <i>T. b. gambiense</i> <i>T. b. rhodesiense</i> <i>T. evansi</i> <i>T. equiperdum</i>	480
<i>Nannomonas</i>	<i>T. godfreyi</i> <i>T. simiae</i> (Tsavo) <i>T. simiae</i> <i>T. congolense</i> (kilifi) <i>T. congolense</i> (savannah) <i>T. congolense</i> (forest)	280 370 400 620 700 700

The PCR protocol published by Njiru *et al.* (2005) was modified slightly by reducing the primer concentration from 1  $\mu$ M to 0.4  $\mu$ M to minimize the production of non-specific PCR products. Reaction mixtures contained 5  $\mu$ l of eluted DNA, master-mix consisting of 2.5  $\mu$ l of 10X reaction buffer (670 mM Tris-HCl pH 8.8, 166 mM  $(\text{NH}_4)_2\text{SO}_4$ , 4.5% Triton X-100, 2 mg/ml gelatin) (Bioline, UK), 1  $\mu$ l of 50mM  $\text{MgCl}_2$ , (Bioline, UK), 0.2  $\mu$ l of 25mM (dNTPs) (Rovalab, Germany), 1  $\mu$ l of 10 pmol/ $\mu$ l forward and reverse primers and 1  $\mu$ l of 1U/ $\mu$ l BioTaq DNA polymerase (Bioline, UK). The final reaction volume of 25  $\mu$ l was obtained by adding 14.3  $\mu$ l of double distilled water (ddH<sub>2</sub>O). Thermal cycling was carried out in a Bio-Rad Dyad Peltier thermal cycler (MJ Research Inc., USA) and cycling conditions consisted of an initial denaturation step at 94°C for 5 min, followed by 35 cycles of 94°C for 40 seconds, 58°C for 40 seconds, and 72°C for 90 seconds with final extension at 72°C for 5 min. Negative controls included in each PCR run consisted of the blank filter paper punch extract and 5  $\mu$ l of PCR master-mix. Purified DNA known to be positive for *T. vivax* was used as positive control.

### 3.5.2 Gel electrophoresis

About 15  $\mu$ l of the PCR products was loaded onto a 1.5% (w/v) agarose gels stained with 0.04  $\mu$ l/ml of GelRed™ nucleic acid stain (Biotium Inc., USA) and resolved by electrophoresis in 1-hour runs at 110V in 1X Tris-borate–EDTA (TBE) buffer. A 100-bp molecular ladder (exACTGene™) (Fisher Scientific, UK) was loaded along with samples on each gel to determine PCR fragment sizes. Results were viewed and documented using a UV trans-illuminator (Gel-Doc™ 2000) with the Quantity One software (Bio-Rad Laboratories, Inc.).

### **3.6 Data Analysis**

Apparent density of tsetse or mean tsetse catch per trap was calculated as total number of tsetse trapped, divided by the number of traps multiplied by number of trapping days (FAO, 2008). The point prevalence of trypanosome infections at each sampling point was determined as the percentage of animals infected with exact binomial 95% confidence intervals included. Cumulative incidence of trypanosome infections at each sampling point was calculated as the proportion of new infections relative to the uninfected population. Fisher's exact test was used to compare differences in the proportion of animals infected with trypanosomes over the whole study period in relation to age group (adult versus juvenile) and sex (male versus female) and P-values < 0.05 were considered to be significant. Analyses were carried out using graphpad®, an online statistical analysis software available at [www.graphpadstats.com](http://www.graphpadstats.com).

### **3.7 Results**

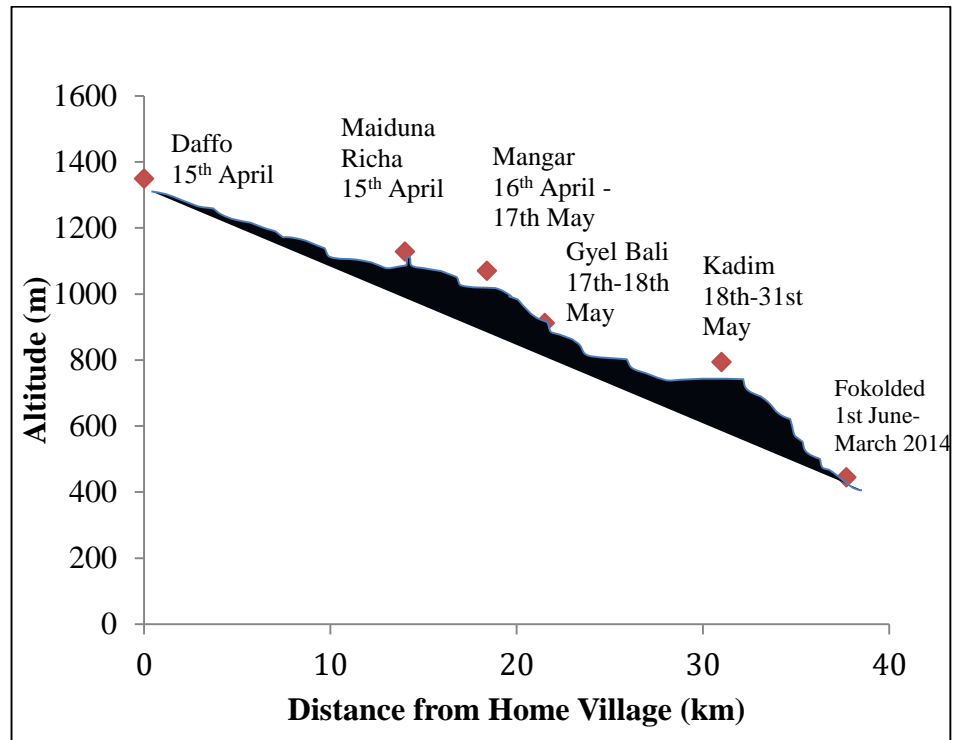
#### **3.7.1 The selected herd and its migration- Daffo to Fokolded**

The studied herd comprised 120 cattle (74 females and 46 males) and is normally resident in the village of Daffo in Bokkos LGA and. The village is located between latitude 9.26°N and longitude 8.82°E at an average altitude of 1270m. The landscape is dominated by open grassland, cultivated fields and few scattered trees (Figure 3.1). A few fragments of riverine vegetation providing limited shade on stream and river banks are present.



**Figure 3.2: The study herd in its home village-Daffo.**

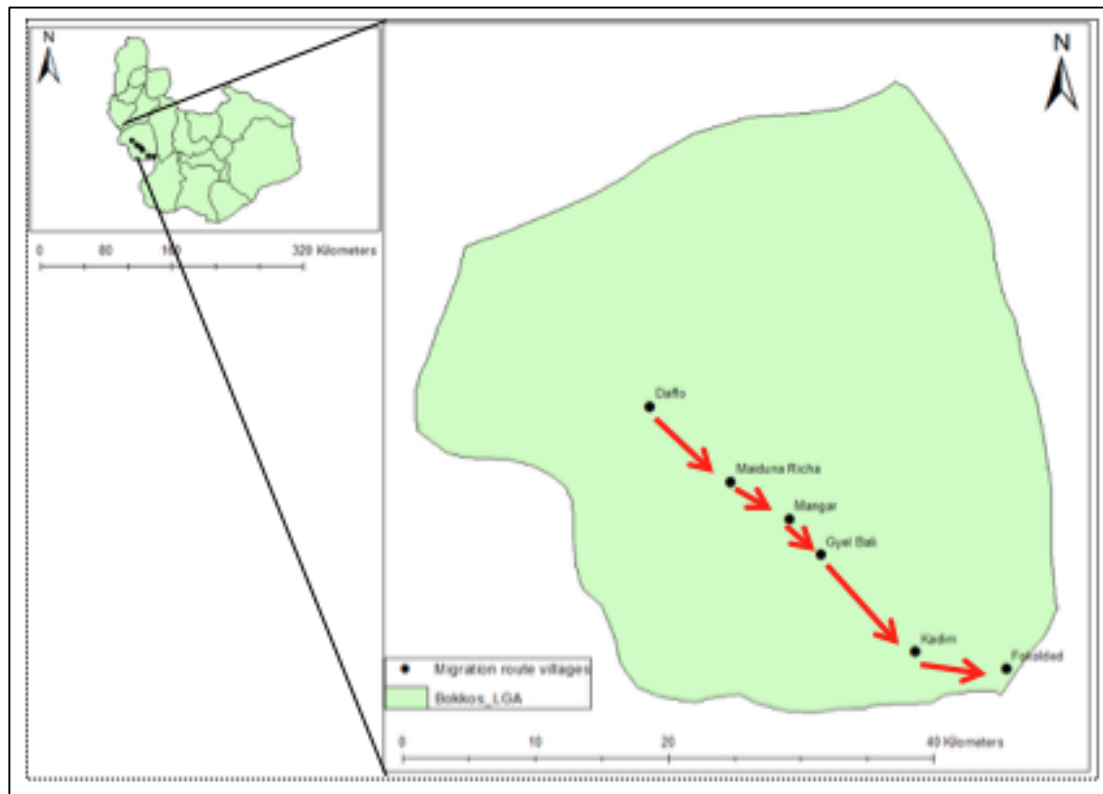
The animals left Daffo on the 15<sup>th</sup> of April 2013 herded by three of the owner's younger brothers and reached Maiduna Richa on the same day. They spent one night in this village and the next day, proceeded to Mangar where they camped and spent two weeks grazing the animals. On the 1<sup>st</sup> of May, they left Mangar and moved about 3 kilometres to Gyel-Bali where they stopped and grazed the herd for one day before proceeding to Kadim where they spent two weeks. They arrived in Fokolded on the 1<sup>st</sup> of June where they remained for about nine months. Figure 3.2 illustrates the progression of the journey showing the distance travelled and the altitudinal gradient from Daffo to Fokolded.



**Figure 3.3: Progression of migration from Daffo and dates at each location.**

A central location in each village along the route was marked using a GPS Oregon450® (Garmin, USA) and shown on maps of Plateau State and Bokkos LGA in Figure 3.4.





**Figure 3.4: Map of Plateau State and Bokkos LGA showing migration route from Daffo to Fokolded.**

There were some differences in the land cover and land use patterns in these villages; Maiduna Richa was characterised by intensive cultivation and much of the natural vegetation showed signs of human activities such as clearance for farmland and firewood collection. There was a reduction in the extent of such activities in Mangar which was even more marked in Gyel Bali which had dense patches of riverine vegetation and many areas of uncultivated grassland with a few farms. In Kadim there were vast areas of closed gallery forests and some *Isobertinia* woodland which was fragmented by farmland. Monkeys and baboons were observed in the gallery forests in this village and a hunter was seen carrying a porcupine which had been

trapped in the bushes around the village. The landscape in Fokolded was dominated by open grasslands with sparse wood cover and some riverine vegetation. All of the villages were characterised by the presence of rocky outcrops covered by grasses, shrubs and small trees on which cattle were grazed especially at the peak of the wet season (August) when the surrounding plains sometimes became flooded. Figures 3.5 – 3.8 illustrate some of the features of the villages described above.



**Figure 3.5: Fragmented Landscape and wood harvesting in Maiduna Richa.**



**Figure 3.6: Dense Riverine Vegetation in Gyel Bali.**



**Figure 3.7: Landscape in Kadim showing gallery forests on hillsides and in valleys.**





**Figure 3.8: Cattle grazing in an area of Fokolded with sparse tree cover.**

### **3.7.2 Tsetse species and apparent density**

During the trap surveys conducted along the migration route, 48 *G. p. palpalis* were trapped during the first round of sampling in Maiduna Richa, Gyel Bali, Kadim and Fokolded. An additional 32 flies of the same species were trapped in Gyel Bali during a second visit in August, bringing the total number of tsetse trapped to 80. Table 3.4 provides a summary of the numbers of flies trapped per village and the apparent density of tsetse in each village based on number of tsetse, traps and trapping days.

**Table 3.4: Apparent density of tsetse (*G. p. palpalis*) and time spent by herd in sampled villages**

Village (month sampled)	Duration of Herd's stay	Number of Traps	Number of Flies	Apparent Tsetse Density (Number of flies/trap/ day)
Maiduna Richa (July)	1 day	10	1	0.03
Gyel Bali (July)	1 day	12	38	1.05
Gyel Bali (August)	1 day	12	32	0.89
Kadim (July)	2 weeks	10	7	0.23
Fokolded (July)	9 months	10	2	0.07

As determined by a longitudinal trap survey previously described in Chapter 2, tsetse flies are not present in the home village- Daffo, which lies at a mean altitude of about 1270 m above sea level. However, from Maiduna Richa where the altitude is about 1200 m, tsetse flies are present and their density peaks in Gyel Bali which is at an altitude of 900 m. From observations made during sampling, it appears that availability of suitable vegetation (riverine habitats) is more important than altitude, in limiting tsetse presence and density in these villages.

### **3.7.3 Trypanosome Infections in Cattle**

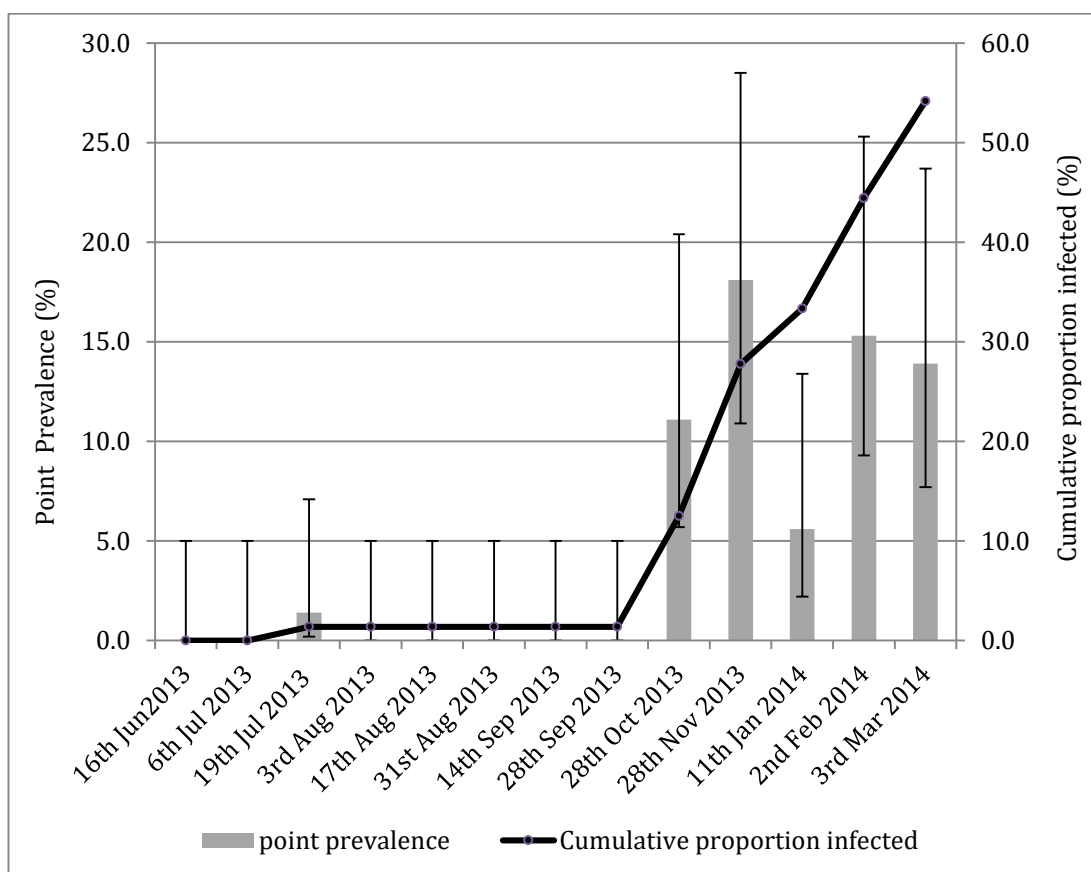
#### **3.7.3.1 Overall incidence of trypanosome infections**

Over a period of nine months between June 2013 and March 2014, blood samples were obtained at 13 different time points from 72 cattle which had migrated from Daffo to Fokolded. Screening of the samples for trypanosomes by PCR revealed that 54.2% i.e. 39 of the 72 animals (95% CI: 42.0 – 66.0%) had become infected with *T. vivax* during the period of the study and no other trypanosome species was detected

in any of the blood samples at all sampling points. The rate of infection among female cattle was 24/41 or 58.5% (95% CI: 42.1% – 73.7%) and among male cattle, it was 15/31 (48.4%; 95% CI: 30.2% - 66.9%). Analysis of the results using Fisher's exact test showed that the difference in proportion of male and female cattle infected was not statistically significant ( $p = 0.48$ ). Age group also had no significant effect on trypanosome infection ( $p = 0.81$ ) as juveniles (< 2 years old) and adults (> 2 years old) had similar infection rates; 52.0% (31.3% - 72.2%) and 55.3% (40.1% - 69.8%) respectively.

#### **3.7.3.2 Point prevalence and cumulative incidence of *T. vivax* infections**

The point prevalence of trypanosome infection (*T. vivax*) in the sampled cattle varied between sampling points for the duration of the study. It was 0.0% (95% CI: 0.0 – 5.0%) at the first two sampling points in mid-June and early-July, rose only slightly to 1.4% (95% CI: 0.2% – 7.1%) in mid-July and dropped back to 0.0% at each of the five sampling points from August to the end of September. Between October 2013 and March 2014, prevalence at each of the sampling points ranged between 5.6% and 18.1% and the cumulative proportion of animals infected which had remained low from June to September, rose sharply in this period (Figure 3.9).



**Figure 3.9: Point Prevalence and Cumulative Proportion of trypanosome infections in 72 cattle sampled during migration from Daffo to Fokolded from 16th June 2013 – 3rd March 2014.**

(Error bars represent 95% confidence intervals).

### 3.7.3.3 Inconsistent detection of *T. vivax* infections

In 33 of the 39 animals found to be positive for *T. vivax*, during the course of the study, infections were not detected at any time point after the initial detection at an earlier sampling point. Only in six of the 39 infected animals were infections detected at one or more subsequent sampling points after the first detection and records for these are shown in Table 3.5.

**Table 3.5: Infected animals in which *T. vivax* was detected more than once over the sampling period.**

Animal ID	Sex	Age Group	28 <sup>th</sup> Oct	28 <sup>th</sup> Nov	11 <sup>th</sup> Jan	2 <sup>nd</sup> Feb	3 <sup>rd</sup> Mar
85b	Female	Adult	+	+	-	+	-
69y	Female	Adult	+	-	-	+	-
83g	Male	Adult	-	+	-	+	-
82b	Female	Juvenile	-	-	+	-	+
88o	Female	Adult	-	-	+	-	+
88b	Male	Adult	-	-	-	+	+

+ Positive; - Negative; Oct = October, 2013; Nov = November, 2013; Jan = January, 2014; Feb= February, 2014; Mar = March, 2014.

### 3.8 Discussion

During the past three decades, trypanosomiasis has emerged as an important disease of cattle on the Jos Plateau in north central Nigeria despite the low density of the tsetse fly vectors of trypanosomes in this area. Seasonal migration of Fulani cattle, which in recent times has become more frequent and of longer duration, has been suggested to be a major risk factor for bovine trypanosomiasis on the plateau but this had not been determined empirically. Therefore, to investigate the role played by seasonal migration of cattle in the transmission and spread of AAT in this area, the migration of a herd of cattle along a cattle migration route from Daffo to Fokolded in Bokkos LGA was followed.



### **3.8.1 Migration pattern**

The studied herd undertook a wet season migration in April 2013 descending from an altitude of around 1300 m in Daffo to about 420 m above sea level in Fokolded having moved a distance of approximately 40 km. This movement did not exceed the boundaries of Bokkos LGA still on the plateau although the final location of the herd was right at the base of the plateau. The distance moved by this herd is within the range of Fulani seasonal movements on the plateau reported by Awogbade (1979). According to the farmer, the distance moved could have been even shorter except for the need to avoid areas of dense human settlement and intensive cultivation so as to forestall conflict. This suggests that the need to avoid conflict is a major factor affecting migration patterns. The herd reached Fokolded on the 1<sup>st</sup> of June and remained there for the duration of the wet season and the ensuing dry season although it was to have returned to Daffo in October. When probed as to why the herd did not return as planned, the farmer's response was that there was an abundance of grazing in Fokolded without any trouble over crop damage.

Also, the trek from Daffo to Fokolded lasted 32 days, as the herd stopped and grazed at various locations along the way for periods ranging from one day to four weeks. This, as well as the farmer's decision to extend the stay in Fokolded beyond October, illustrate the point made by Blench (1994) that seasonal Fulani migrations are often more complex than is shown on maps with arrows indicating direction of movement or distance travelled. He suggested that migratory movements and the duration of stay at different locations depended on prevailing conditions such as availability of

grazing or the need to avoid disease outbreaks or vectors. This suggestion applied to the studied herd as will be later illustrated.

### **3.8.2 Tsetse presence and relative abundance**

An entomological survey using unbaited biconical traps revealed the presence of only *G. p. palpalis* at densities ranging from 0.03 - 1.05 flies per trap per day in the villages along the migration route. This again confirms *G. p. palpalis* as the predominant tsetse species on the Jos Plateau; a fact evident in previous studies dating back to Taylor (1930) and as recently as chapter two of this thesis. Riverine vegetation which is the preferred habitat of this tsetse (Nash, 1969; Nash & Page, 1953) was abundant in some of the villages sampled, especially Gyel-Bali and Kadim where human settlements were few and widely dispersed. The vegetation in both villages was relatively undisturbed as there were few, mostly rain-fed farms which did not encroach on river banks.

The suitability of the habitat for tsetse especially in Gyel Bali was reflected by the higher apparent tsetse densities (1.05 flies per trap per day in July) and (0.89 flies per trap per day in August) recorded in this village compared to 0.2 in Kadim, 0.23 in Fokolded and 0.03 in Maiduna Richa. In Maiduna Richa which had the lowest apparent tsetse density, the landscape was extremely fragmented as a result of farming and woodcutting for firewood. It was expected that Kadim, like Gyel-Bali, would also harbor a high density of flies because of its extensive gallery forests and woodlands. However, the apparent density of tsetse here was almost five times less

than it was in Gyel Bali. It is possible that the flies were widely dispersed in the extensive forests and woodlands and thus not many were trapped. More intensive trapping using a higher number of traps and over a longer period could provide different and probably more accurate estimates of tsetse density in these areas.

The importance of vegetation as a component of suitable habitat for riverine tsetse has been previously demonstrated. For example, Bourn (1983) found a positive association between the size of riverine vegetation patches and the presence and abundance of *G. p. palpalis* in Lafia just below the escarpment of the Jos Plateau. Similarly, Bouyer *et al.* (2005) also found that fragmentation of riverine forest had a negative effect on the presence and density of riverine tsetse in Burkina Faso. It is, however, known that apart from vegetation, many other factors such as altitude, temperature, humidity and the availability of host animals contribute to the suitability of habitats for tsetse (Nash, 1969; Rogers and Randolph, 1993) and these are likely to have played a part in the results obtained in the respective villages.

Only two flies were trapped in Fokolded and this was a surprising result as habitat conditions in some parts of this village were similar to those in Gyel-Bali even though overall, the habitat was more fragmented in Fokolded. However, it is possible that the weekly deltamethrin spray employed by the farmer for tick control had led to a reduction in tsetse density in Fokolded. It has been shown that deltamethrin-treated cattle can be used as an effective means of tsetse control especially in areas with high cattle densities and where cattle are the main hosts of tsetse (Bauer *et al.*, 1999; Torr

*et al.*, 2005). It is very likely that cattle are the main hosts of tsetse in the surveyed area where there are hardly any wild animals and many migratory herds from other parts of the country spend a significant proportion of the year.

As indicated in the last paragraph of sub section 3.8.1, the migration strategy adopted by the owner of the studied herd, appears to be influenced by prevailing ecological conditions and it is interesting to note that it seems to take into particular consideration, the observed variations in tsetse density/challenge along the route. Thus, while the herd spent two weeks grazing in Mangar, where based on results from chapter two, the apparent tsetse density is relatively low (0.03 flies per trap per day), it spent just one day in Gyel Bali where the high density of tsetse would have caused a high biting nuisance and posed a higher risk of trypanosome infections. Kadim and Fokolded where the herd spent a combined period of nine months spanning the almost the entire length of the wet and dry seasons, both had similarly low tsetse densities. The short time spent in Maiduna Richa seems to contradict this line of reasoning since this village had the lowest apparent tsetse density. However, a possible explanation for the short stay could have been the presence of many farms; which resulted in a limited availability of grazing areas.

Although the above interpretation of events is mostly speculative, it is supported by information from the herd owner that the herd was only grazed for longer periods in Gyel Bali during the dry season when there was a reduction in tsetse numbers and the flies became confined to certain locations which were avoided as much as possible.

However, since such sites of fly concentration coincide with the only available water source during the late dry season, contact between fly and cattle is inevitable and as such, animals are likely to become infected with trypanosomes during such periods.

### **3.8.3 Trypanosome Infections in Cattle**

#### **3.8.3.1 Seasonal variation in transmission risk**

The results obtained from the cattle blood samples indicate that there is a lower risk of AAT transmission during the wet season compared to the dry season. Throughout the wet season from June to September, only one animal was found to have been infected with *T. vivax* which was the only trypanosome species detected. The remaining 38 infections occurred during the dry season between October and March.

This result seems to be in contrast to the general trend in which trypanosome transmission risk rises during the wet season as tsetse numbers increase and the flies become more widely dispersed (Leak *et al.*, 1999; Nash & Page, 1953). As indicated by the low number of tsetse recorded in Fokolded where the herd was stationed, tsetse may have been few and widely dispersed and this could have resulted in less contact between the vectors and the cattle. Another explanation for the low incidence of infections during the wet season could have been the prophylactic dose of isometamidium administered to the cattle by the farmer in April. This drug has been shown to provide effective protection against trypanosome infections for up to three months under conditions of suppressed or low tsetse challenge (e.g. 0.5 flies/trap/day) (Magona *et al.*, 2004). This was the situation in Fokolded where based on

biconical trap catches, tsetse density was even lower at 0.06 flies per trap per day and the first infection was detected in mid-July about three months after the prophylactic treatment was administered.

After the first infection was detected, no further infections were detected in any of the examined samples until October, which marked the beginning of the dry season. By that time, the effects of the drug would have worn off and it is likely also, that the tsetse population had become more concentrated around permanent pools shaded by riverine vegetation. Such sites provide the microhabitat conditions necessary for tsetse survival during the dry season when most rivers dry up and form isolated pools under shade (Nash and Page (1953). In addition, the cattle were watered more frequently (up to five times each day) at such pools during the dry season and would have been exposed to more tsetse bites which resulted in higher rates of transmission.

#### **3.8.3.2 The complete dominance of *T. vivax***

The only trypanosome species detected in the sampled cattle was *T. vivax* and this might be expected in conditions such as obtained in Fokolded i.e. low density of *G. palpalis* known for its efficiency as a vector of West African (Nigerian) strains of *T. vivax* (Moloo *et al.*, 1987). This result also suggests that *G. p. palpalis* in this area takes most of its blood meals from a bovid host (in this case, cattle) since this trypanosome is commonly found in Bovidae (Jordan, 1965). Cattle from the plateau and nearby Nasarawa and Bauchi States are present in the area surveyed on a year round basis, and are likely to provide the majority of tsetse blood meals as there are

no reports of wild Bovidae in the area and none were seen during tsetse or cattle sampling.

Early studies of *T. vivax* in Nigeria (Lester, 1933, and Unsworth, 1953 cited by Mwongela, 1981) showed that Nigerian strains of this trypanosome were highly virulent and often caused severe mortality in zebu cattle. Although the animals in this study were not monitored for clinical signs, they appeared to be in good condition and no deaths were recorded throughout the duration of the study. It has been suggested that even though zebu cattle do not have innate resistance to trypanosome infections, animals of this breed which survive trypanosome infections with or without chemotherapy subsequently become more resistant to infection (Murray *et al.*, 1981). This gradual acquisition of some degree of resistance is attributed to the development of immunity to local trypanosome populations in a particular area and this may apply to the herd in this study.

#### **3.8.3.3 Overall incidence, point prevalence and fluctuating parasitaemia**

Despite the fact that between July 2013 and February 2014, *T. vivax* had been detected at least once in 32 (~44%) of the 72 sampled animals, only three (9.2%) of these 32 infections were detected at the last sampling point in March during which the point prevalence was 13.1% (11/72 infected). A similar trend was observed at all sampling points except in October when point prevalence (11.1%) was almost equal to the cumulative proportion of infected animals (12.5%). This is because as pointed out earlier, 33 of 39 (84%) of all infections were detected just once even though the

animals were sampled several times over the period of study between June and March. Indeed, at all sampling points from October till March, many old infections went undetected and this shows that point prevalence may likely underestimate the actual prevalence of infections.

The failure to detect infections consecutively could have been due to low and fluctuating parasitaemia. Thus, following initial detection, parasitaemia in most of the animals may have fallen intermittently to levels that were not detectable even by the sensitive PCR technique employed. Indeed, Masake *et al.*, (1977) noted that detection of *T. vivax* in cattle is sometimes difficult because parasitaemia in many infections is often very low. In contrast to this, Godfrey *et al.* (1965) found that *T. vivax* infections in a herd of cattle trekked along a trade route were characterized by high parasitaemia and were detectable over a long period. However, it must be pointed out that the animals in the latter study (i.e. Godfrey *et al.*, 1965) were trekked about 415 miles under almost continuous challenge from highly infected *G. m. submorsitans* for a considerable part of the journey. The animals would have been stressed by the journey and were probably exposed to more pathogenic trypanosome strains which could explain why parasitaemia was high and sustained.

It is thus possible that the animals in the current study exhibited low parasitaemia since they were not particularly stressed and the tsetse fly challenge was low. Also, as mentioned earlier, prolonged exposure of zebu cattle to trypanosome infections in areas with a history of extensive use of trypanocides, leads many animals to develop



some measure of resistance which enables them to control parasitaemia and remain in good condition unless subjected to nutritional or physical stress (Murray *et al.*, 1982). Under such conditions, some animals have been known to self-cure and infections are no longer detectable unless the animal receives a new infection (Murray, 1989). The animals in this study herd certainly fall into the category of animals previously exposed to both trypanosome infections and trypanocides as most had migrated between Daffo and Fokolded for up to three years and had received trypanocides during the period. Besides, grazing was relatively abundant except for the latter part of the dry season in February and March.

An additional reason for the non-detection of infections could be the use of blood samples stored on FTA cards. The trypanosome DNA is not evenly distributed across the card matrix and as such, infections in blood samples of animals with low grade parasitaemias are likely to escape detection for this reason as clearly demonstrated by (Cox *et al.*, 2010).

Finally, the possibility that animals which appeared sick were treated with a trypanocide by the farmer or his brothers who had charge of the herd during the course of the study cannot be ruled out. Infected animals may have shown a loss of condition which farmers have been known to interpret as a sign of trypanosome infections prompting the application of curative medicines (Majekodunmi *et al.*, 2013; Okello *et al.*, 2014). The treatments could have completely cured the animals or reduced the parasitaemia such that infections were not detectable. Although the

farmer stated that he did not treat his animals with a trypanocide, it is possible that one of the herd boys may treated the cattle without the owner's knowledge.

#### **3.8.3.4 Absence of *T. brucei* and *T. congolense***

Despite conditions which would favor a *T. vivax*-dominated epidemiology (i.e. low density of an efficient *T. vivax* vector (*G. palpalis*), it was still expected that other species of trypanosomes such as *T. brucei* and *T. congolense* would have been detected in the studied herd although at much lower rates relative to *T. vivax*. This expectation is based on the findings of previous AAT surveys on the plateau (e.g. Kalejaiye *et al.*, 2004; Majekodunmi *et al.*, 2013) during which these species were detected alongside *T. vivax*. Besides, it was thought that the other herds present in the area; most of which were from Nasarawa State where *G. tachinoides* and possibly some *G. m. submorsitans* might be present, would have introduced these trypanosome species into the *G. palpalis* population present in Fokolded. However, since migrant herds from other areas were not sampled, this can only be assumed, although examination of the trapped *G. palpalis* for trypanosomes could provide clarification and this will be carried out in the next chapter.

### **3.9 Summary and Recommendations**

This study was limited by its small sample size i.e. one herd among many on the plateau, studied along one of several migration routes. Furthermore, the measure taken by the owner of the herd to protect his animals primarily against ticks and tick-borne diseases by spraying them weekly with deltamethrin is likely to have affected

the apparent tsetse density in Fokolded and thus, the overall incidence of trypanosome infections in the herd. However, despite these limitations, the study provides important insights on seasonal migration and the transmission of AAT to cattle on the Jos Plateau.

Firstly, it demonstrates that *G. p. palpalis* is present along the studied cattle migration route and is the main or sole vector in this area. Secondly, it shows that a significant proportion (54.2%; 95% CI: 42.0 - 66%) of cattle migrating according to the pattern observed in the selected herd could become infected with trypanosomes (in this case, *T. vivax*) during migration. Thirdly, it illustrates that there are spatial variations in transmission risk due to differences in tsetse densities in the areas through which cattle migrate. Risk also varies over time and transmission seems to be higher during the dry season than the wet season although wet season transmission may have been affected by farmer interventions. Frequent and close contact between tsetse and cattle as both converge on permanent water pools on shaded streams as the dry season progresses could be the main reason for the high rate of dry season transmission.

The study also provides some useful information on the knowledge that pastoralists have about the transmission of AAT. It showed that the owner of the studied herd was aware of the variations in risk over time and space as seen in the migration strategy adopted. However, he seems to assume that the risk of transmission is less during the dry season following a decline in the number of tsetse he observed

attacking the animals and hence, preventative measures such as the spraying of the cattle with deltamethrin were halted. Nevertheless, his actions illustrate that farmers could be quite knowledgeable about the risks associated with migration and have developed relatively effective strategies to minimize such risks. This farmer's willingness to adopt new strategies developed through formal research is seen in his use of weekly deltamethrin sprays for tick control; a method which he only recently learnt from the SOS pilot study referred to in Chapter One. Such a farmer could serve as an important link between researchers and other Fulani cattle owners on the Jos Plateau in collaborative efforts to develop an effective and sustainable community-based strategy for the control of AAT in the area.

It is further acknowledged that the current study provides only some part (albeit an important one) of the answer to the question of how migration contributes to widespread bovine trypanosomiasis on the Jos Plateau. To answer this question more adequately, investigations of other migration routes and migrating herds from the plateau are needed; to clarify the extent to which trypanosomes are introduced to the plateau through seasonal migration. Comparison of the strains of *T. vivax* in non-migrating herds with those in migrating herds could enable the discovery of differences in virulence between local and introduced strains. Future studies should involve a larger sample involving several migrating herds, appropriate controls comprising herds which do not migrate, continuous observations of all study herds with regard to daily and seasonal activity patterns and periodic blood sampling for trypanosomes accompanied by more intensive entomological surveys.

## **4 Trypanosomes and Endosymbionts in Tsetse Flies from the Jos Plateau and Yankari Game Reserve, Bauchi State**

## 4.1 Introduction

Both sexes of tsetse feed exclusively on vertebrate blood and all tsetse species are potentially capable of the cyclical transmission of trypanosomes which cause human and animal trypanosomiasis in sub-Saharan Africa (SSA). *Trypanosoma brucei gambiense* and *T. b. rhodesiense* (subgenus *Trypanozoon*) cause chronic and acute African human sleeping sickness respectively (Welburn *et al.*, 2001) while *T. vivax* (subgenus *Duttonella*), *T. congolense*, *T. simiae* (sub-genus *Nannomonas*), and *T. b. brucei* (subgenus *Trypanozoon*) are the main causative agents of animal African trypanosomiasis (AAT) which leads to significant morbidity, loss of productivity and mortality in a broad range of domestic livestock (Reifenberg *et al.*, 1997).

Tsetse flies can only acquire trypanosomes from their vertebrate hosts, and so, tsetse hosts and feeding patterns play an important role in the epidemiology of trypanosomiasis. Analysis of data from several studies on tsetse hosts and trypanosome infection rates in wild tsetse from different parts of Nigeria by Jordan (1965) showed that the trypanosome infection rate and dominant trypanosome species in tsetse depended largely on their main hosts. He observed that *Glossina* species which took most of their blood meals from wild fauna (Bovidae or Suidae) had more trypanosome infections than those which fed on domestic animals; the rates were highest for those tsetse species in which the main hosts were wild Bovidae. While *T. vivax* occurred more frequently in tsetse that fed mainly on Bovidae while the rate of occurrence of *T. congolense* did not seem to vary much between those that fed on Bovidae and those which fed on Suidae (Jordan, 1965).

Many of the natural hosts of tsetse and trypanosomes are wildlife species such as bushbuck (*Tragelaphus scriptus*), waterbuck (*Kobus ellipsyprimmus*), warthog (*Phaenoceros aethiopicus*), impala (*Aepyceros melampus*), and the common duiker (*Sylvicapria grimmia*), which are immune to the pathogenic effects caused by trypanosomes in humans and domestic stock (Ashcroft, 1959). These wild hosts often serve as reservoirs of trypanosomes (Anderson *et al.*, 2011; Auty *et al.*, 2012) that can be transmitted by tsetse to hosts such as man and domestic animals. The influence of tsetse hosts or blood meal sources on the nature and extent of trypanosome infections is attributed to their differing capacities as reservoirs of trypanosomes (Jordan, 1965).

However, transmission of trypanosomes acquired from a blood meal is preceded by a period of cyclical development in different parts of the tsetse alimentary tract that affects trypanosome infection rates in tsetse and the risk of transmission (Lindh and Lehane, 2008). *T. vivax* has a relatively simple cycle which takes place only within the tsetse mouthparts and is completed in about five to 13 days (Bruce *et al.*, 1911 in Stephen, 1986; Lloyd & Johnson, 1924). Trypanosomes of the subgenera *Nannomonas* (e.g. *T. congolense*) and *Trypanozoon* (e.g. *T. brucei*) undergo more complex cycles in tsetse; first they become established as procyclics in the ectoperitrophic space of the tsetse midgut and subsequently migrate to the proboscis and salivary glands respectively, where they mature into transmissible metacyclic forms (Gibson and Bailey, 2013; Peacock, 2012a, 2012b).

In *T. congolense*, development in tsetse is completed in about 14 days but may range from 7-40 days (Dale *et al.*, 1995; Hoare, 1970) while *T. brucei* takes around 30 days varying between 7 and 45 days in extreme cases (Hoare, 1970). The difference in the complexity and time it takes each of these trypanosome species to develop within the tsetse host is related to the rates at which they occur in wild tsetse. As such, tsetse flies are often easily infected by, and harbour more infections of *T. vivax* than *T. congolense* or *T. brucei* in which the process is progressively more complex and longer (Jordan, 1986). This is of course moderated by the rates of occurrence of each trypanosome species in the hosts utilised by tsetse (Jordan, 1965).

Observations made in laboratory experiments confirm that many tsetse flies are highly refractory to mid-gut establishment of *T. brucei* and *T. congolense* infections (Maudlin and Welburn, 1994). Additionally, ingested bloodstream trypanosomes are often eliminated before they are able to establish a procyclic infection in the ectoperitrophic space and a further proportion of flies in which procyclic midgut infections establish, are able to prevent maturation to salivary gland or mouthpart infections (Maudlin and Welburn, 1994, Maudlin 1991). These factors account for the low rates of mature *T. congolense* and *T. brucei* infection rates in wild tsetse population even when tsetse are exposed to many infected hosts. For example, Jordan (1974) found that only 10% of 9000 field-dissected tsetse belonging to the *morsitans* group (which includes some of the most efficient vectors of trypanosomes) harboured mature infections of any of the three salivarian trypanosome subgenera but especially of *T. brucei*; despite the fact that the flies had been trapped from different



parts of Nigeria where wildlife hosts known to be reservoirs of trypanosomes were relatively abundant (Jordan, 1974).

Further investigations have revealed that many factors are involved in the refractoriness or susceptibility of tsetse to trypanosome infections, and that these are in operation throughout the development of trypanosomes through tsetse. They include external factors such as the climatic conditions in which tsetse develop, and intrinsic fly-related factors such as species, age, sex, teneral state, nutritional status, immune response, genetic composition, and the presence of endosymbionts (reviewed by Roditi & Lehane, 2008). The combined effects of these factors affect the susceptibility of tsetse to trypanosomes and ultimately determine infection rates and subsequent transmission of trypanosomes.

#### **4.1.1.1 Endosymbionts and trypanosome infections in tsetse**

Certain maternally-inherited endosymbiotic bacteria present in tsetse flies have been shown to be involved in interactions which affect the establishment and maturation of trypanosome infections in tsetse (Maudlin and Welburn, 1998; Aksoy *et al.*, 2003). They include the obligate or primary (P)-symbiont *Wigglesworthia glossinidia*, the parasitic *Wolbachia*, and a commensal, secondary (S)-symbiont *Sodalis glossinidius* (Dale and Maudlin, 1999; Ma and Denlinger, 1974 in Aksoy, 1995; O'Neill *et al.*, 1993).

*W. glossinidia*, a member of the Enterobacteriaceae is transmitted to the intrauterine larva via maternal milk glands and is found in specialized epithelial cells known as bacteriocytes which form a U-shaped bacteriome in the anterior mid-gut of adult tsetse (Aksoy, 1995). Phylogenetic studies demonstrate that *W. glossinidia* displays concordant evolution with its host species in an association which is about 50-80 million years old (Chen *et al.*, 1999). Its streamlined genome of about 700 kb encodes a variety of vitamins and biosynthetic products that promote tsetse reproduction and nutrition throughout its life cycle (Akman *et al.*, 2002; Rio *et al.*, 2012). The absence of this symbiont renders female tsetse sterile, although this can be partially reversed by supplementation of the blood meal with vitamin B, reversal is only partial suggesting that the symbiont may have additional functional roles which cannot be replaced by vitamin supplementation (Nogge, 1981). With regard to its role in tsetse-trypanosome interactions, recent work has shown that *Wigglesworthia* is important to the proper development of tsetse immune system during larval development (Weiss *et al.*, 2012).

*Wolbachia* is a genus of endosymbiotic  $\alpha$ -proteobacteria infecting a wide range of filarial nematodes and arthropods including insects; and tsetse flies are among many insect species infected by a variety of *Wolbachia* strains (Werren *et al.*, 2008). In many species as well as in tsetse, they cause several reproductive abnormalities including parthenogenesis, male-killing, feminization and cytoplasmic incompatibility (CI) (Alam *et al.*, 2011). Two basic forms of CI are recognised. Unidirectional CI involves one *Wolbachia* strain in which matings between infected

males and uninfected females results in the females producing fewer surviving offspring in comparison to other possible matings (Stouthamer *et al.*, 1999). This is due to an incompatibility between egg and sperm. Bi-directional CI is caused by two *Wolbachia* strains and can occur when mating partners are infected with different strains. Attempts are ongoing to exploit the ability of *Wolbachia* to manipulate tsetse reproduction for the purpose of driving traits such as refractoriness to trypanosomes into wild tsetse populations (Aksoy *et al.*, 2008).

While *Wolbachia* has not been shown to directly affect tsetse-trypanosome interactions, there is much interest in taking advantage of its ability to modify reproductive outcomes in its hosts to decimate, as well as drive anti-trypanosome traits into natural populations of tsetse (Doudoumis *et al.* 2013). Studies have thus been undertaken to determine its prevalence in laboratory reared and natural populations of tsetse. Doudoumis *et al.* (2012) showed that for laboratory-reared tsetse, an infection rate of 100% is common but the rate of infection varies widely in wild populations depending on the tsetse species and their geographical origins. They studied *Wolbachia* infections in 3750 tsetse (551 specimens from six different laboratory stocks and 3199 from natural populations) of nine *Glossina* species from 10 African countries using a *Wolbachia* specific 16S rRNA-based PCR assay. *Wolbachia* was prevalent in *G. m. morsitans*, *G. m. centralis* and *G. austeni* populations. It was also detected in *G. brevipalpis* and for the first time in *G. pallidipes* and *G. p. gambiensis* from Mali and Senegal but not in *G. p. palpalis*, *G. tachinoides* and *G. f. fuscipes*; suggesting that perhaps the *palpalis* group do not

harbour *Wolbachia* infections although the reasons for this are unclear (Doudoumis *et al.* 2012).

*Sodalis glossinidius*, the secondary symbiont of tsetse, is also a member of the Enterobacteriaceae whose association with tsetse is more recent but it has received more attention with regard to the susceptibility of tsetse to trypanosomes. In studies of laboratory-reared tsetse (Geiger *et al.*, 2005; Maudlin and Dukes, 1985), this symbiont was found to increase the susceptibility of tsetse to trypanosome infections with *S. glossinidius*-positive flies having significantly higher rates of trypanosome infections.

Such an association has also been detected in field-caught *G. palpalis* (Farikou *et al.*, 2010) and *G. pallidipes* (Wamwiri *et al.*, 2013). On the other hand, Dennis *et al.* (2014) did not find an association between *S. glossinidius* and trypanosome infections in *G. brevipalpis*, *G. m. morsitans* and *G. pallidipes* trapped in Zambia. Geiger *et al.* (2007) have further shown that the genotype of *S. glossinidius* rather than its mere presence is more important in increasing the susceptibility of *G. p. gambiensis* to midgut infections of *T. b. gambiense* and *T. b. brucei*. The prevalence of *S. glossinidius* in laboratory flies often approaches 100% but is highly variable in natural populations. For example, prevalence of *S. glossinidius* in *G. brevipalpis*, *G. m. morsitans* and *G. pallidipes* from Zambia was 93.7% (95% CI: 82.7 - 97.1) 17.5% (95% CI: 12.1 – 24.7) and 1.4% (95% CI: 0.8 – 1.5) respectively (Dennis *et al.*, 2014).

#### 4.1.2 Identification of trypanosomes in tsetse

Traditionally, trypanosomes in tsetse were identified following the method developed by Lloyd & Johnson (1924). This involves dissection and microscopic examination of the fly mouthparts, salivary glands and midgut for trypanosomes. Identification of trypanosomes was based on which of these organs were infected; trypanosomes present only in the mouthparts were identified as *T. vivax*, those infecting mouthparts and midgut were categorised as *T. congolense*-type infections while trypanosomes detected in midgut and salivary glands were identified as *T. brucei*. Infections confined to the midgut were considered to be immature *T. brucei* or *T. congolense*. This method is however limited by its inability to differentiate species within the subgenus *Nannomonas* and the subspecies of *Trypanozoon*, and inability to detect mixed infections.

Recently, the application of more sensitive molecular techniques, has led to the detection of higher infection rates in wild-caught tsetse; an infection rate of 40.7% was reported in *G. tachinoides* and *G. p. palpalis* from north-central Nigeria (Majekodunmi, 2006) while Morlais *et al.* (1998) also reported an infection rate of 53.8% for *T. congolense* in 21/52 PCR positive *G. p. palpalis* trapped in Cote d'Ivoire. More recently, Malele *et al.* (2011) showed that with dissection, studies produced lower levels of detection than PCR which showed much higher levels of detection. Using dissection the prevalence of *T. brucei* s.l. was 2.5% while with PCR it was found to be 55%. This is a 22 fold difference. Dennis *et al.* (2014) suggested that more work might need to be done in terms of using PCR to diagnose infections

in wild flies as it is unclear whether the PCR is picking up an active infection in the fly, or dying parasites imbibed with the bloodmeal.

## 4.2 The current work

This overall objective of this thesis is to provide additional understanding of the factors that have led to the spread and transmission of AAT transmission on the Jos Plateau. In this area where AAT which was once rare (Pullan, 1980), Majekodunmi *et al.* (2013) report that the disease is now widely prevalent with an overall prevalence of 46.8% (CI: 39.0% - 54.5%) Although they recorded a low prevalence of *T. brucei* (3.2%), *T. congolense* was very prevalent and accounted for 27.7% of infections; this was slightly higher than the prevalence of *T. vivax* at 26.7%. The current high levels and wide spatial spread of AAT on the plateau have been attributed mainly to the somewhat recent invasion of the plateau by tsetse flies which are thought to have over time become widely distributed. It is also speculated that seasonal migration of cattle to areas with higher levels of tsetse is leading to the introduction of trypanosome infections on to the plateau. Chapters two and three of this thesis, have examined these two factors that are thought to be largely responsible for the current state of AAT on the plateau.

In Chapter 2, entomological surveys conducted in 20 villages on the Jos Plateau revealed that *G. p. palpalis* though present, had a limited distribution (present in two of 20 villages) while *G. tachinoides* was found in just one village. These results do not support suggestions that tsetse distribution is expanding on the plateau.

Results from Chapter 3 provide strong evidence that seasonal migration of cattle owned by Fulani agro-pastoralists on the Jos Plateau is an important means by which trypanosome infections are introduced onto the plateau. By following a herd of cattle migrating from an area of high altitude (~1300 m) on the plateau where tsetse flies were absent, it was confirmed that *G. p. palpalis* was present along the herd's migration route and at its destination. Subsequent examination of blood samples obtained from the herd also showed that during the 10 months spent at this location, 54.2% (44.0 – 66%) of the sampled animals became infected with *T. vivax*.

Although the results from the previous chapters of this thesis may explain the presence of *T. vivax* on the plateau, they do not account for the especially high prevalence of *T. congolense* and the limited presence of *T. brucei* found by (Majekodunmi *et al.*, 2013). The presence, in a few locations on the plateau, of *palpalis* group tsetse which are not very efficient vectors of *T. congolense* and *T. brucei*, and the fact that only *T. vivax* was detected in the migrant herd in chapter 3, do not quite tally with the findings of Majekodunmi *et al.* (2013) although it is acknowledged that in the period between the two studies, changes may have occurred with respect to the trypanosomes circulating within the vector and consequently, cattle populations on the plateau. It is therefore important to determine the trypanosomes circulating within tsetse on the Jos Plateau as a measure of the risk of AAT and to see if there is any correlation to the results found in cattle.

Very few studies have been conducted to determine trypanosome infection rates in tsetse on the plateau. Dede *et al.* (2005) reported an overall infection rate of 1.67% due to *T. brucei* and *T. vivax* based on the dissection of 240 tsetse flies (114 *G. tachinoides* and 126 *G. palpalis*) from the plateau. All the infections were detected in the *G. tachinoides* specimens while the *G. palpalis* were free of trypanosomes. Kalu (1991) recorded three mouthpart-only infections likely to be *T. vivax* from dissections of 28 *G. tachinoides* and in another study in the same area, Kalu (1996a) found that one of nine (11.1%) trapped *G. tachinoides* harboured a *T. vivax* infection while none of five *G. p. palpalis* dissected were infected.

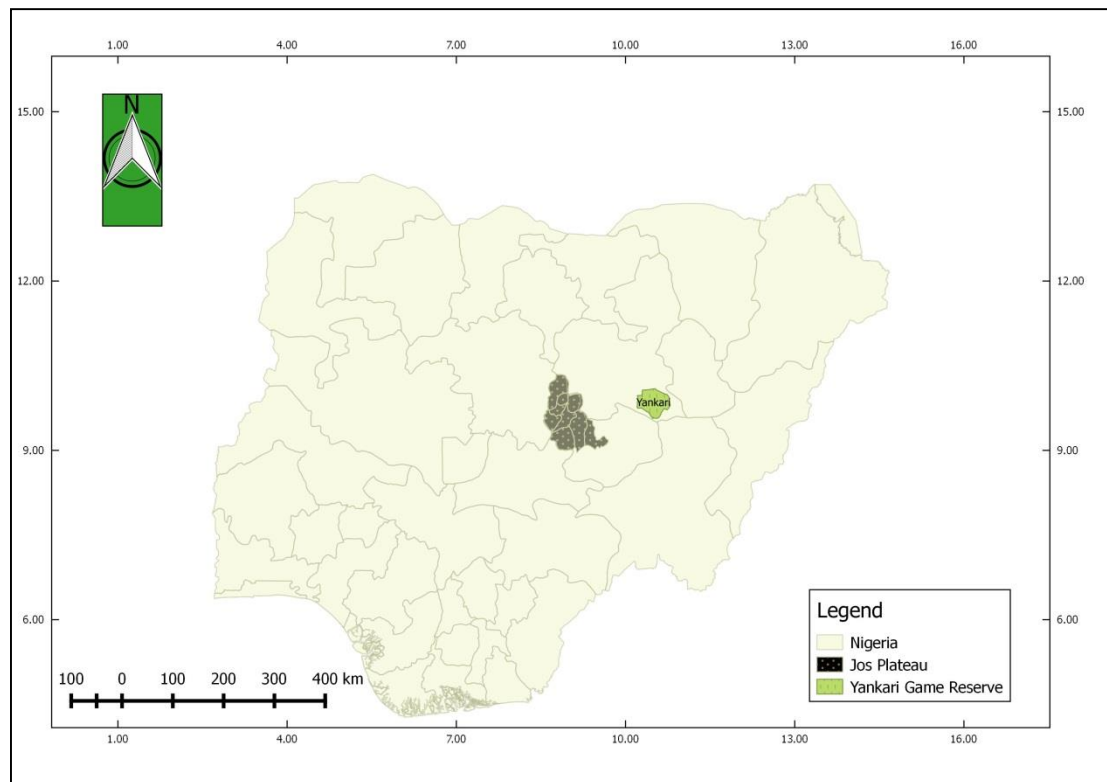
Majekodunmi (2006) used both microscopy and molecular techniques (ITS-PCR) developed by Adams *et al.*, (2006) to identify trypanosome infections in 140 tsetse flies (31 *G. palpalis* and 109 *G. tachinoides*) from four villages on the plateau. The overall infection rate as determined by the more sensitive PCR technique was 40.7% although there was a discrepancy between the dissection and PCR results as seen in other studies using both methods (Morlais *et al.*, 1998; Masiga *et al.*, 1992). The prevalence according to the trypanosome species encountered were *T. congolense* (11.8%), *T. brucei* (10.3%), *T. vivax* (8.8%), *T. godfreyi* (27.9%) and *T. simiae/simiae* (Tsavo) (41.2%) (Majekodunmi, 2006). This study was not only the first to report such a high infection rate of trypanosomes in tsetse on the plateau, but was also the first to report the presence of *T. simiae/T. simiae* (Tsavo) and *T. godfreyi* (which could not have been identified by dissection). These results were



probably due to the more sensitive and specific molecular technique of trypanosome detection employed.

Generally, results from dissection of tsetse are less reliable than the more sensitive and specific molecular PCR assays now available for the detection of trypanosome infections in tsetse and their vertebrate hosts (Njiru, *et al.*, 2004; 2005). But assuming there was no contamination and the findings of Majekodunmi (2006) are accurate, they demonstrate that it is possible for *palpalis* group tsetse to be highly infected with some trypanosome species although it could not be determined whether the detected infections were viable and transmissible infections. Nevertheless, in view of recent findings of high AAT prevalence on the plateau, and considering that the most recent study of trypanosomes in tsetse was conducted almost 10 years ago, it is important to provide more up to date information on trypanosomes circulating in tsetse in this area in order to determine the risk to cattle.

The current study is an investigation of the trypanosomes and endosymbionts, of tsetse flies trapped on the Jos Plateau in north central Nigeria, and in Yankari game Reserve which is located in Bauchi State south east of the Jos Plateau about 130 km from Jos, the Plateau State capital (Figure 4.1).



**Figure 4.1: Map of Nigeria showing the location of Yankari Game Reserve relative to the Jos Plateau**

Dispersal of tsetse along rivers linking Yankari Game Reserve to the plateau has been suggested to be a possible route through which tsetse invaded the plateau (Majekodunmi *et al.*, 2013a) although this has not been proven. A stronger connection may be found in the movement of cattle between the two areas as recently, Blench (2004) observed that some Fulani pastoralists migrated from the plateau and settled temporarily in the areas surrounding the reserve in the aftermath of violent conflicts on the Jos Plateau. Omondi *et al.* (2006) confirm that Fulani pastoralists have been observed grazing their cattle within the boundaries of the reserve.

The increasing frequency and length of seasonal cattle migration from the plateau to areas where tsetse flies are present, and temporary settlement of Fulani in places near Yankari Game Reserve in nearby Bauchi State (Blench, 2004) where both tsetse and wildlife are known to be abundant, more than one species of trypanosome was expected to be circulating within the surveyed herd. Hence, it would be of considerable epidemiological interest, to examine tsetse flies from the plateau and possibly Yankari Game Reserve, to determine their trypanosome infection rates as well as the species of trypanosomes they harbour; the results of which could shed more light on what is known about the transmission, spread and current state of AAT on the Jos Plateau.

### **4.3 Chapter Aim and Objectives**

The overall aim of this thesis is to provide a better understanding of tsetse and the transmission of AAT on the Jos Plateau in order to inform the choice of strategies for the control of the disease in this area. The aim of this particular chapter is to determine the diversity and infection rates of trypanosome species infecting tsetse flies collected from the Jos Plateau and the Yankari Game Reserve in neighbouring Bauchi State as a measure of the risk tsetse pose to cattle in these areas. The prevalence of *S. glossinidius*, a secondary bacterial endosymbiont present in some tsetse and known to increase the susceptibility of tsetse to trypanosomes is also investigated. The flies are also screened for the presence of *Wolbachia*; a parasite of tsetse reproductive organs which has not been previously reported in natural populations of *G. p. palpalis*. This is the first study of endosymbionts in tsetse from

these areas and is expected to contribute to what is known about the prevalence of endosymbionts in wild tsetse populations. The specific objectives are:

1. To determine the species of trypanosomes and their prevalence in tsetse flies trapped from the Jos Plateau and Yankari Game Reserve, Bauchi State.
2. To determine the prevalence of *S. glossinidius* and test if there is an association between the presence of *S. glossinidius* and trypanosome infections in the examined tsetse flies.
3. To determine if *Wolbachia*, which has not been reported from *G. p. palpalis* elsewhere, is present in this species on the Jos Plateau or in the other species of tsetse (*G. m. submorsitans* and *G. tachinoides*) found in Yankari Game Reserve.

## **4.4 Materials and Methods**

This section provides some information on the origins of tsetse fly samples screened and the methods by which they were collected preserved, transported and processed in order to meet the stated objectives. It also describes the methods used to analyse the data generated.

### **4.4.1 Origin of Tsetse Fly Samples**

The tsetse fly samples used in these investigations are from various sources/locations on the Jos Plateau and the Yankari Game Reserve as shown in Table 4.1.

**Table 4.1: Sources of tsetse fly samples showing date of collection, species and numbers of male and female flies**

Source/Location	Date of collection	Tsetse species	No. of flies (Male:Female)
Mangar, Jos Plateau	April 2012 - Sept 2013	<i>G. palpalis</i>	30 (13:17)
Assop, Jos Plateau	July 2013	<i>G. palpalis</i>	41 (20:21)
Angware, Jos Plateau	July 2013	<i>G. tachinoides</i>	1 (1:0)
Maiduna Richa, Jos Plateau	July 2013	<i>G. palpalis</i>	1 (1:0)
Gyel Bali, Jos Plateau	July/August, 2013	<i>G. palpalis</i>	70 (51:19)
Kadim, Jos Plateau	July 2013	<i>G. palpalis</i>	7 (5:2)
Fokolded, Jos Plateau	July 2013	<i>G. palpalis</i>	2 (2:0)
Yankari Game Reserve, Bauchi State	May - July 2012	<i>G. palpalis</i>	49 (27:22)
		<i>G. tachinoides</i>	73 (36:37)
		<i>G. morsitans</i>	58 (22:36)
		Total	332 (178:154)

Study locations, trapping, identification and preservation methods have been previously described in Chapters 2 and 3 for flies originating from villages on the Jos Plateau (longitudinal and cross-sectional surveys Chapter 2; migration study Chapter 3). The flies from Yankari Game Reserve were generously donated by colleagues at

the A. P. Leventis Ornithological Research Institute (APLORI) University of Jos, Nigeria. They were collected using unbaited biconical traps set within riverine and savannah habitats in the reserve between May and July, 2012. All fly samples were shipped to the Infectious Diseases Laboratories, Division of Pathway Medicine of the University of Edinburgh under import licences PATH 176/2012/7 and PO/2013/03 and stored at -80°C until required for DNA extraction.

Yankari Game Reserve is located between Latitude 9°4' and 10°00' N and Longitudes 10°17' and 10°47'E and occupies an area of about 2 244 km<sup>2</sup> of savannah woodland in a shallow basin that slopes from West, North and East towards the centre and South. The altitude ranges between 200 m – 640 m (Tende *et al.*, 2011). Similar to the Jos Plateau, rainfall in Yankari Game Reserve is highly seasonal and under the influence of two wind systems; the south west monsoonal winds which prevail during the rainy season between May and September and the north-east trade wind during the dry season. Average rainfall ranges from 900-1000mm with August as the wettest month. Temperatures are moderate between 18°C and 33°C during the rainy season. During the dry season, between November and February, night temperatures are between 12°C and 18°C while day temperatures range from 30°C and 36°C. Between March and April, night temperatures range between 25°C and 30°C while day temperatures are between 38 °C and 42 °C (Tende *et al.*, 2011).

The reserve hosts an abundance of wildlife species that include buffalo (*Syncerus caffer*), roan antelope (*Hippotragus equinus*), waterbuck (*Kobus ellipsiprymnus*), western hartebeest (*Alcelaphus buselaphus*), bushbuck (*Tragelaphus scriptus*), oribi (*Ourebia ourebi*), hippopotamus (*Hippopotamus amphibius*), and lion (*Panthera leo*). While some species, such as buffalo and roan antelope appear to be relatively numerous in the reserve, others (such as hippopotamus and lion) are rarely observed and may be approaching extirpation (Tende *et al.*, 2011). Over the last 50 years, species such as giraffe (*Giraffa camelopardalis*), cheetah (*Acinonyx jubatus*) and wild dog (*Lycaon pictus*) have been eliminated from the reserve but despite these losses, Yankari remains the most intact example of savannah fauna in Nigeria (Tende *et al.*, 2011). *G. m. submorsitans*, *G. tachinoides* and *G. p. palpalis* are the three tsetse fly species present within the reserve (Clement *et al.*, 2013).

The reserve is currently under severe pressure from human activity and like many protected areas in West Africa, it is an ecological island; surrounded almost completely by human habitation and areas of intense cultivation. While agricultural encroachment on the reserve itself is minimal, illegal cattle grazing is a significant problem. Livestock are frequently driven into the reserve to graze causing competition with wildlife for food, bringing disease (rinderpest outbreaks have significantly reduced wildlife populations in the past (Nawathe and Lamorde 1983), and causing habitat destruction through the cutting of browse and seasonal bush burning (Omondi *et al.*, 2006).

#### **4.4.2 DNA Extraction**

Each fly was thawed and surface sterilized in a 5% solution of bleach. It was then rinsed twice in phosphate buffered saline (PBS) and left to dry on a piece of filter paper at room temperature. The wings and legs were removed and the head with the mouthparts attached was detached from the thorax and abdomen using a sterile scalpel (Swann-Morton, Sheffield, UK). The detached head and mouthparts of each fly were placed in a sterile 1.5ml micro centrifuge tube while the abdomen was placed in a separate tube so that DNA from these parts were extracted separately.

The Qiagen DNeasy® blood and tissue kit (QIAGEN Inc., USA) was used for the extractions following the manufacturer's instructions. Briefly, samples were crushed using a sterile, autoclaved polypropylene pestle followed by the addition of 180 µl of lysis buffer and 20 µl of proteinase K. Samples were then incubated overnight in a water bath at 56°C to allow complete lysis of the cells. Following this, 200 µl of buffer (AL buffer) containing a chaotropic salt and 200 µl ethanol were added to each sample to provide optimal DNA binding conditions. The preparation was mixed by vortexing and transferred to a DNeasy® mini spin column. This was followed by micro centrifugation during which the DNA is bound to the silica membrane of the spin column and two wash steps to remove contaminants and potential PCR inhibitors. Finally, the DNA was eluted in 200 µl of elution buffer and stored at -20°C until required for PCR reactions.



DNA was extracted separately from mouthparts and abdomens to differentiate between trypanosomes present in the gut and those in the mouthparts and to allow some inference to be made on the status of infections. Based on knowledge of the development of the trypanosome groups in tsetse, the detection of trypanosome DNA in either or both of these separated parts may be used to infer the status of infection as follows: the detection of *T. congolense* or other *Nannomonas* DNA in only the gut of a fly is indicative of an immature infection whereas *T. vivax* in the same location clearly is of no importance as it has no chance of becoming mature. *T. vivax* DNA in the mouthparts only, would indicate a developing or mature infection while *T. congolense* DNA in both mouthparts and abdomen would suggest a mature infection. Inference cannot be made about *T. brucei* as detection of DNA of this trypanosome in the abdomen could either be from a gut or salivary gland infection since the salivary glands extend into the abdominal cavity. However, it is important to note that detection of trypanosome DNA might just be the result of bloodmeal contamination and not of an active infection within the tsetse (Dennis *et al.* 2014)

#### **4.4.3 PCR for *Wigglesworthia glossinidia***

All fly DNA extracts were first screened for *W. glossinidia* the primary endosymbiont found in all tsetse flies in order to confirm the success of the DNA extraction process. Primers were used to amplify a 129bp fragment of the *flagellin* (FliC) gene of this endosymbiont (Soumana *et al.*, 2013). Standard PCR reactions took place in 25 µl volumes containing 1 µl of eluted DNA, 1X reaction buffer (670 mM Tris-HCl pH 8.8, 166 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 4.5% Triton X-100, 2 mg/ml gelatin) (Bioline, UK), 2mM MgCl<sub>2</sub>, (Bioline, UK), 0.2mM of each dNTP (Rovalab,

Germany), primers at a concentration of 0.1 µM and 1Unit of BioTaq DNA polymerase (Bioline, UK). Cycling conditions consisted of an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles of 94°C for 40 seconds, 58°C for 40 seconds, and 72°C for 90 seconds with final extension at 72°C for 5 minutes. Thermal cycling for this and all other PCRs were carried out in a Bio-Rad Dyad Peltier thermal cycler (MJ Research Inc., USA).

Only samples positive for *W. glossinidia* were screened by additional PCR assays to determine the prevalence of trypanosome infections, *S. glossinidius* and *Wolbachia*. The PCR targets, primer sequences and expected product sizes for all PCR assays are shown in Table 4.2.

**Table 4.2: PCR Targets, Primer Sequences and Expected band sizes**

PCR Target	Primer	Sequence (5'-3')	Expected product sizes (bp)	Reference
<i>Wigglesworthia glossinidia</i>	FliC-F FliC-R	TATACGAGGATCTTTAGGAGC GATACTTCTGTGGCAAAATCTGC	129	Soumana <i>et al.</i> (2013)
<i>Trypanosoma</i> spp. ITS1 rDNA	ITS1-CF ITS1-BR	CCGGAAGTTCACCGATATTG TTGCTGCGTTCCTCAACGAA	250-720 depending on species and sub species	Njiru <i>et al.</i> (2005)
<i>Sodalis glossinidius</i>	SodGRoEl-F SodGroEl-R	CCAAAGCTATCGCTCAGGTAGG TTCTTTGCCCACTTTCGCCATA	95	Matthew <i>et al.</i> (2005)
<i>Wolbachia</i>	ftsZ_F1 ftsZ_F2	ATYATGGARCATATAAARGATAG TCRAGYAATGGATTTRGATAT	524	Baldo <i>et al.</i> (2006)
	wsp 81F wsp 691R	TGGTCCAATAAGTGATGAAGAAAC AAAAATTAAACGCTACTCCA	610	Chen <i>et al.</i> (2000)

A positive control consisting of DNA known to be positive for each specific PCR was included in each run and double distilled water (ddH<sub>2</sub>O) was used as a negative control. DNA from a teneral colony-raised *G. p. palpalis* was included as negative extraction control in the ITS-1 PCR runs.

#### **4.4.3.1 Detection of trypanosomes by ITS-1 PCR (Njiru *et al.*, 2005)**

Trypanosome infections in the tsetse samples were detected using the generic ITS-1 primers and protocol of Njiru *et al.* (2005), which enables the simultaneous detection of all known pathogenic species of African trypanosomes. The procedure and cycling conditions have been described in section 3.5.1 of Chapter 3.

#### **4.4.3.2 PCR for the detection of *Sodalis glossinidius***

Flies were screened for *S. glossinidius* using primers that amplify a 95 bp fragment of the GroEl gene (Matthew *et al.*, 2005). Reaction volume was 25 µl and contained 1 µl of eluted DNA, 1X reaction buffer (670 mM Tris-HCl pH 8.8, 166 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 4.5% Triton X-100, 2 mg/ml gelatin) (Bioline, UK), 1.5 mM MgCl<sub>2</sub>, (Bioline, UK), 0.2mM of each of the four deoxy-nucleotide triphosphates (dNTPs) (Roalab, Germany), primers at 0.4µM and 1Unit of BioTaq DNA polymerase (Bioline, UK). Cycling conditions were: initial denaturation at 94°C for 5 minutes followed by 35 cycles of 94°C for 40 seconds, 58°C for 40 seconds and 72°C for 90 seconds. A final extension step was carried out at 72°C for 5 minutes.

#### **4.4.3.3 PCR for *Wolbachia***

A pair of primers that amplify a 524bp fragment of the *ftsZ* gene (Baldo *et al.*, 2006) and a second pair, which amplify a 500bp fragment of the *Wolbachia* surface protein (*wsp*) gene (Cheng *et al.*, 2000) were used in two separate PCR assays to screen fly DNA samples for the presence of *Wolbachia*. Both reactions were carried out in 25 µl volumes containing 1X reaction buffer (670 mM Tris-HCl pH 8.8, 166 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 4.5% Triton X-100, 2 mg/ml gelatin) (Bioline, UK), 1.5mM MgCl<sub>2</sub>,

(Bioline, UK), 0.2mM of each of the four (dNTPs) (Rovalab, Germany), primers at 0.5  $\mu$ M and 1Unit of *BioTaq* DNA polymerase (Bioline, UK). Cycling included an initial denaturation step at 94°C for 5 min followed by 35 cycles of 94°C for 40 s, 58°C for 40 s, 72°C for 90 s, and final extension at 72°C for 5 minutes.

#### **4.4.4 Gel electrophoresis and visualization of PCR products**

All PCR products were resolved by electrophoresis on 1.5% (w/v) agarose gels stained with 0.04  $\mu$ l/ml of GelRed™ nucleic acid stain (Biotium Inc., USA) and run for 45 to 70 minutes at 100V in 1X Tris-borate–EDTA (TBE) buffer. A 100-bp molecular ladder (exACTGene™) (Fisher Scientific, UK) was loaded along with samples on each gel to determine PCR fragment sizes. Results were viewed and documented with a UV trans-illuminator (Gel-Doc™ 2000) using the Quantity One software (Bio-Rad Laboratories, Inc.).

### **4.5 Data Analysis**

Trypanosome prevalence was calculated as the percentage of flies in which trypanosome DNA was detected either in the gut, mouthparts or both gut and mouthparts. The prevalence rate of endosymbionts was also computed as the percentage of flies in which endosymbiont DNA was detected. Confidence intervals (95%) were calculated for these percentages and differences in infection and prevalence rates between male and female flies and between villages were tested for significance using Fisher's exact test and p-values less than 0.05 were considered

significant. The analyses were done using graphpad; a free online statistical software available at (<http://graphpad.com/quickcalcs/index.cfm>).

## 4.6 Results

DNA extracted from 332 tsetse flies trapped on the Jos Plateau and from Yankari Game Reserve were screened for trypanosomes to assess the trypanosomiasis risk to cattle. Flies were also screened for *S. glossinidius* the secondary endosymbiont of tsetse which may increase tsetse fly susceptibility to trypanosomes and for *Wolbachia*, a parasite of tsetse reproductive organs.

An initial PCR for *W. glossinidia* the obligate endosymbiont present in all tsetse flies was used to determine the success of the DNA extraction process. Only samples positive for this symbiont underwent further screening for trypanosomes and the other endosymbionts and were included in the calculations of infection and prevalence rates. Fly DNA samples negative for *W. glossinidia* were not included in further PCR or statistical analysis. The total number of male and female tsetse of all species from all sampling locations that were positive for *W. glossinidia* was 303 as shown in Table 4.3.

Table 4.3: *Wigglesworthia glossinidia*-positive tsetse fly samples

Source/Location	Tsetse species	Number screened (M : F)	<i>Wigglesworthia</i> -positive (M : F)
Mangar, Jos Plateau	<i>G. palpalis</i>	30 (13 : 17)	30 (13 : 17)
Assop, Jos Plateau	<i>G. palpalis</i>	41 (20 : 21)	41 (20 : 21)
Angware, Jos Plateau	<i>G. tachinoides</i>	1 (1 : 0)	1 (1 : 0)
MaidunaRicha, Jos Plateau	<i>G. palpalis</i>	1 (1 : 0)	1 (1 : 0)
Gyel Bali, Jos Plateau	<i>G. palpalis</i>	70 (51 : 19)	67 (50 : 17)
Kadim, Jos Plateau	<i>G. palpalis</i>	7 (5 : 2)	7 (5 : 2)
Fokolded, Jos Plateau	<i>G. palpalis</i>	2 (2 : 0)	2 (2 : 0)
Yankari Game Reserve, Bauchi State	<i>G. palpalis</i>	49 (27 : 22)	38 (19 : 19)
	<i>G. tachinoides</i>	73 (36 : 37)	64 (31 : 33)
	<i>G. morsitans</i>	58 (22 : 36)	52 (18 : 34)
	Total	332 (178 : 154)	303 (160 : 143)

M:F= Ratio of male to female flies

## 4.6.1 Trypanosome infections

### 4.6.1.1 Jos Plateau-overall and village level prevalence

Of the 149 *W. glossinidia*-positive flies (148 *G. palpalis* and one *G. tachinoides*) from the Jos Plateau, trypanosome DNA was detected in 79 flies giving a prevalence of 53% (95% CI: 44.7- 61.2%). One male *G. palpalis* had *T. congolense* DNA in its

mouthparts only, while all the other 78 trypanosome-positive flies were infected by *T. vivax*. Thus, *T. vivax* accounted for (98.7%, 95% CI: 93.1 – 100%) of the total infections while *T. congolense* prevalence was (1.3%, 95% CI: 0.0% – 6.9%). No other trypanosome species were detected in the screened flies. The prevalence of infections in the villages from which flies were collected with exact binomial confidence intervals is shown in Table 4.4.

**Table 4.4: Trypanosome (*T. vivax*) prevalence in tsetse from villages on the Jos Plateau**

Village	Number of flies examined	Number of flies with trypanosome DNA (%)	95% Confidence Intervals for proportion infected (%)
Mangar	30	9 (30.0)	16.5 – 48.0
Assop	41	26 (63.4)	46.9 – 77.9
Angware	1*	1 (100.0)	2.5 – 100.0
Maiduna Richa	1	0 (0.0)	0.0 – 97.5
Gyel Bali	67	36 (55.2)	28.5 – 53.0
Kadim	7	5 (71.4)	29.0 – 96.6
Fokolded	2	2 (100.0)	15.8 – 100.0

\*The single tsetse from Angware was a male *G. tachinoides* while all tsetse flies from the other villages were *G. p. palpalis*.

Difference in trypanosome prevalence was significant between Assop and Mangar ( $p = 0.015$ ) and between Assop and Gyel Bali ( $p = 0.029$ ). All other village by village comparisons were not significant. Results of the analysis using Fisher's exact method are shown in Table 4.5.



**Table 4.5: Comparison of trypanosome (*T. vivax*) prevalence in tsetse from villages on the Jos Plateau (\*indicates significant difference at  $p < 0.05$ )**

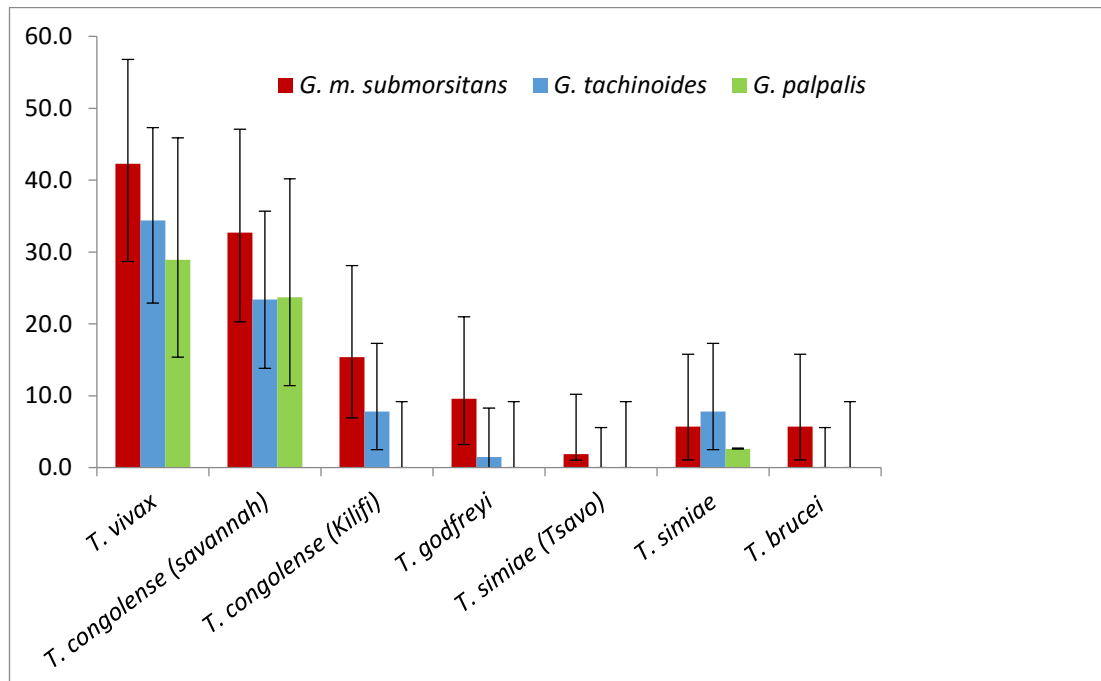
Village	Mangar	Assop	Angware	M/Richa	Gyel Bali	Kadim	Fokolded
<b>Mangar</b>		0.015	1	1	0.496	0.090	0.126
<b>Assop</b>	0.015*		1	1	0.029*	1	0.535
<b>Angware</b>	1	1		1	0.412	1	1
<b>M/Richa</b>	1	1	1		1	0.375	0.333
<b>Gyel Bali</b>	0.496	0.029	0.412	1		0.228	0.173
<b>Kadim</b>	0.090	1	1	0.375	0.228		1
<b>Fokolded</b>	0.126	0.535	1	0.333	0.173	1	

\*Significant at  $p < 0.05$ .

#### **4.6.1.2 Trypanosome prevalence in flies from Yankari Game Reserve**

Trypanosome prevalence in the 154 flies (38 *G. palpalis*; 64 *G. tachinoides* and 52 *G. m. submorsitans*) from Yankari Game Reserve was as follows: *G. palpalis* 44.7% (95% CI: 28.6% – 61.7%); *G. tachinoides* 53.1% (95% CI: 40.2% – 65.7%) and *G. m. submorsitans* 57.6% (43.2% - 71.3%). Trypanosome prevalence did not differ significantly between *G. p. palpalis* and *G. tachinoides* ( $p = 0.53$ ); *G. p. palpalis* and *G. m. submorsitans* ( $p = 0.28$ ) or between *G. tachinoides* and *G. m. submorsitans* ( $p = 0.71$ ).

The prevalence of the various trypanosome species detected in the flies from Yankari is shown in Figure 4.2 which shows *T. vivax* and *T. congolense* (Savannah) to be the most prevalent trypanosomes in the three fly species.



**Figure 4.2: Prevalence of trypanosome species detected by ITS-PCR in *G. m. submorsitans*, *G. tachinoides* and *G. p. palpalis* from Yankari Game Reserve, Bauchi State, Nigeria.** Error bars show 95% confidence intervals.

#### 4.6.1.3 Potentially transmissible infections

Separate PCR reactions were performed on tsetse mouthparts and on abdomens (containing tsetse guts) in order to estimate the proportion of potentially transmissible infections. Mouthpart-only *T. vivax* infections were considered to be mature and transmissible infections. The presence of *T. vivax* DNA in both gut and mouthparts of a fly was classified as a recently acquired infection in which case the trypanosomes in the mouthparts had the potential to mature while those in the gut would be destroyed. Thus by summing mouth-part only infections to those in the

second category (gut and mouthparts) an estimate of potentially transmissible infections was derived and expressed as a proportion of the total number of flies examined (Table 4.6).

**Table 4.6: Prevalence of *T. vivax* infections within the guts and mouthparts of tsetse flies from the Jos Plateau screened by PCR showing estimated prevalence of potentially transmissible infections.**

Village	No. of flies screened	<i>Tv</i> -g	<i>Tv</i> -m	<i>Tv</i> -gxm	Proportion of Potentially Transmissible Infections <i>Tv</i> -m+ <i>Tv</i> -gxm (95% CI)
Mangar	30	1	0	7	23.3% (11.5 – 41.2)
Assop	41	9	12	5	41.5% (26.3 – 57.9)
Angware	1*	0	1	0	100.0% (2.5 – 100.0)
M/Richa	1	0	0	0	0.0% (0.0 – 97.5)
Gyel Bali	67	21	9	7	23.9% (22.3 – 37.5)
Kadim	7	2	0	3	42.9% (9.8 – 81.6)
Fokolded	2	2	0	0	0.0% (0.0 - 84.2)
Total	149	35 (23.5)	22 (14.8)	22 (14.8)	44/149 29.6% (22.3 – 37.5%)

\*The fly was a male *G. tachinoides* while all other flies were *G. palpalis*

*Tv*-g = *T. vivax* gut-only; *Tv*-m = *T. vivax* mouthparts-only; *Tv*-gxm = *T. vivax* gut and mouthparts; Numbers in columns *Tv*-g, *Tv*-m and *Tv*-gxm are counts while those in the last column are percentages derived from summing up the counts in columns *Tv*-m and *Tv*-gxm, dividing by number of flies screened in each village and multiplying by 100 with 95% confidence intervals in brackets. M/Richa= Maiduna Richa.

The total proportion of potentially transmissible *T. vivax* infections was estimated to be 29.5% (44/149; 95% CI: 22.3 – 37.5%).

In the three species of tsetse from Yankari Game Reserve, the proportion of potentially transmissible infections as determined by the presence of trypanosome DNA in mouthparts-only or mouthparts and abdomen (*T. vivax*) and mouthparts and abdomen for *Nannomonas* subspecies are presented in Table 4.7.

**Table 4.7: Potentially transmissible infections indicated by the presence of trypanosome DNA in mouthparts (*T. vivax*) and (mouthpart + abdomen) *Nannomonas* subspecies.**

	<i>G. p. palpalis</i>	<i>G. tachinoides</i>	<i>G. m. submorsitans</i>
<i>T. vivax</i>	2.6% (0.01 – 13.8)	6.3% (1.7 – 15.2)	7.7% (2.1 – 18.5)
<i>T. congolense</i> (Savannah)	7.9% (1.7 - 21.4)	6.3% (1.7 - 15.2)	11.5% (4.4 – 23.4)
<i>T. congolense</i> (Kilifi)	0.0% (0.0 - 9.2)	0.0% (0.0 – 5.6)	1.9 (0.05 – 10.2)
<i>T. godfreyi</i>	0.0% (0.0 - 9.2)	0.0% (0.0 – 5.6)	1.9 (0.05 – 15.9)
<i>T. simiae</i> (Tsavo)	0.0% (0.0 - 9.2)	0.0% (0.0 – 5.6)	0.0% (0.0% - 6.8%)
<i>T. simiae</i>	0.0% (0.0 - 9.2)	0.0% (0.0 – 5.6)	0.0% (0.0% - 6.8%)

Figures in brackets are 95% confidence intervals. Number of flies examined = 38 *G. p. palpalis*, 64 *G. tachinoides* and 52 *G. m. submorsitans*.

#### 4.6.1.4 Mixed Trypanosome Infections

None of the flies from the Jos Plateau was found to contain DNA of more than one trypanosome species. However, mixed infections involving two or three trypanosome species in 11 different species combinations were detected in the flies from Yankari. The number of different combinations was two, seven and nine for *G. p. palpalis*, *G. tachinoides* and *G. m. submorsitans* respectively. The most common combination in the three fly species was of *T. vivax* and *T. congolense* (Savannah), which accounted for 48.7% of all mixed infections.

Overall, 39 flies of all three species had mixed infections of which 35 (89.7%) involved combinations of two trypanosome species while 4 (10.3%) involved three trypanosome species. The distribution of the different trypanosome combinations in each of the fly species is shown in Table 4.8.

**Table 4.8: Mixed Trypanosome infections in tsetse from Yankari Game Reserve**

	<i>G. p. palpalis</i>	<i>G. tachinoides</i>	<i>G. m. submorsitans</i>	Total
Tv/Tcs	5	7	7	19
Tv/Ts	0	1	1	2
Tv/Tck	0	1	1	2
Tv/Tg	0	1	2	3
Tv/Tb	0	0	1	1
Tg/Tcs	0	0	4	4
Ts/Tcs	1	1	0	2
Tcs/Tck	0	1	1	2
Tv/Ts/Tb	0	0	1	1
Tv/Ts/Tcs	0	2	0	2
Tv/Tst/Tcs	0	0	1	1
Total	6	14	19	39

Tv = *T. vivax*; Tcs = *T. congolense* (Savannah); Ts = *T. simiae*; Tb = *T. brucei* s.l.  
Tst = *T. simiae* (Tsavo); Tck = *T. congolense* (Kilifi).

#### 4.6.1.5 Sex of fly and trypanosome prevalence

The sample of flies from the Jos Plateau examined for trypanosomes consisted of 57 females and 92 males. The prevalence of trypanosomes in females was 55.4% (44.7% - 65.8%) and 49.1% (35.6% - 62.7%) in males. The observed difference in trypanosome prevalence between female and male flies was not significant.

In the flies from Yankari Game Reserve, overall trypanosome prevalence in female flies of all species was 43.0% (95% CI 32.4% - 54.2%) and 58.8% (46.2% - 70.6%) in male flies. There was no significant difference in trypanosome prevalence between male and female flies ( $p = 0.07$ ).

In *G. p. palpalis*, trypanosome prevalence by sex was 26.3% (9.1% - 51.2%) in females and 63.2% (38.4% - 83.7%) in males. In *G. tachinoides*, prevalence among females was 54.5% (36.4% - 71.9%) and in males, it was 51.6% (33.1% - 69.8%). Trypanosome prevalence in female *G. m. submorsitans* was 52.9% (35.1% - 70.2%) while in males it was 66.7% (41.0% - 86.7%). In all three species, the difference in trypanosome prevalence between male and female flies was not significant.

## 4.6.2 Endosymbionts

### 4.6.2.1 Overall prevalence of *Sodalis glossinidius*

The prevalence of *S. glossinidius* among the 148 *G. p. palpalis* from the Jos Plateau was 68.94% (95% CI: 61.1% - 75.8%). In *Glossina palpalis*, *S. glossinidius* was detected in 36.5% (95% CI: 23.6% - 51.9%) of flies from Assop, 56.7% (95% CI: 39.2% - 72.6%) from Mangar, 94% (95% CI 85.2-98.1) from Gyel Bali, 100% (95% CI 16.8-100) from Maiduna Richa, 57.1% (95% CI 25.0-84.3) from Kadim and 100% (95% CI 29.0-100) from Fokolded. *S. glossinidius* was not detected in the single specimen of *G. tachinoides* from Angware. Fisher's testing showed that Gyel Bali (94.7%) was significantly higher ( $p < 0.001$ ) than both Assop (36.6%) and Mangar (56.7%). No other significant differences were detected (Table 4.9).

**Table 4.9: Comparison of *S. glossinidius* prevalence in tsetse from villages on the Jos Plateau by Fisher's Test. (\*indicates significant difference at  $p < 0.05$ )**

	<b>Assop</b>	<b>Mangar</b>	<b>Gyel Bali</b>	<b>Maiduna</b>	<b>Kadim</b>	<b>Fokolded</b>
<b>Assop</b>		0.147	<0.001*	0.381	0.412	0.151
<b>Mangar</b>	0.147		<0.001*	1.000	1.000	0.502
<b>Gyel Bali</b>	<0.001*	<0.001*		1.000	0.016*	1.000
<b>Maiduna</b>	0.381	1.000	1.000		1.000	1.000
<b>Kadim</b>	0.412	1.000	0.016*	1.000		0.500
<b>Fokolded</b>	0.151	0.502	1.000	1.000	0.500	

Significantly fewer tsetse flies from Yankari Game Reserve were found to contain *S. glossinidius*. In this group of flies, the prevalence of *S. glossinidius* was 7.9% (95% CI: 2%-21.6%), 6.3% (95% CI: 2.1%-15.9%) and 3.9% (95% CI: 0.2-13.7%) for *G. p. palpalis*, *G. tachinoides* and *G. m. submorsitans* respectively. There was no difference in *S. glossinidius* prevalence between the three species at the Yankari Game Reserve.

#### **4.6.2.2 Sex related prevalence of *Sodalis glossinidius***

The prevalence of *S. glossinidius* among female *G. p. palpalis* on the Jos Plateau was 63.2% (49.3 % - 75.6%). This did not differ significantly from the 68.1% (57.5 – 77.5%) observed in male flies. The low number of flies from Yankari Game Reserve

in which *S. glossinidius* was detected did not allow meaningful statistical comparisons to be made.

#### **4.6.2.3 Association between *Sodalis glossinidius* and trypanosome infection**

It was not possible to determine if there was an association between the presence of *S. glossinidius* and trypanosome infections in the flies from the Jos Plateau because *T. vivax* accounted for 98% of trypanosome infections detected. Unlike trypanosomes of the *Trypanozoon* and *Nannomonas* groups, *T. vivax* does not develop in the mid-gut and its development is not known to be affected by the presence or absence of *S. glossinidius*. The endosymbiont was not found in the one fly (male *G. p. palpalis* from Mangar) in which *T. congolense* DNA was detected.

Of the flies from Yankari, three of 38 *G. p. palpalis* harboured concurrent infections of *S. glossinidius* and trypanosomes (*T. congolense*) in the abdomen. Due to this low number of co-infections however, the association between trypanosome gut infection and the presence of *S. glossinidius* was not significant ( $p = 0.106$ ).

#### **4.6.2.4 *Wolbachia***

All the 303 flies examined were negative for *Wolbachia* using both *wsp* and *ftsZ* primers.



## 4.7 Discussion

DNA samples extracted from 149 tsetse flies trapped in villages of the Jos Plateau and 154 tsetse flies from the Yankari Game Reserve were examined by PCR to detect the presence of trypanosomes and endosymbionts and relate this to the transmission of AAT on the Jos Plateau. The results and their implications are discussed under relevant sub-headings.

### 4.7.1 Trypanosome prevalence

#### 4.7.1.1 Overall infection rate and species

The overall prevalence of trypanosomes was 53% in the *G. p. palpalis* from the various villages of the Jos Plateau. Almost all the infections were due to *T. vivax* and only one fly was infected with *T. congolense*. In Yankari Game Reserve, overall trypanosome prevalence was similarly high, with 44.7%, 53.1% and 57.6% detected in *G. p. palpalis*, *G. tachinoides* and *G. m. submorsitans* respectively. *T. vivax* was again the most common species of trypanosome detected in all three species of tsetse, however, in Yankari Game Reserve, likely due to the greater host diversity, there was greater diversity in the species of trypanosome detected. Whereas on the Jos Plateau, cattle are likely to be the main hosts of tsetse as wildlife are scarce on the plateau.

The high prevalence of *T. vivax* is in agreement with the results obtained by Moloo *et al.* (1987) in experiments involving East and West African strains of *T. vivax* and several species of tsetse including four from the *palpalis* group. They showed that

although, infection rates of the East African stocks were low in *G. p. palpalis*, mature infection rates for the West African stocks in this species were as high as 97%. This demonstrates that *G. p. palpalis* could be an important vector of *T. vivax* in West Africa and on the Jos Plateau. Bourn *et al.* (2001) have suggested that in light of rapid environmental change which has led to the elimination of *G. m. submorsitans* and its wild hosts from large areas of Nigeria and some other parts of West Africa, tsetse of the *palpalis* group had become the main vectors of animal trypanosomes in these areas.

The preponderance of *T. vivax* in tsetse in Jos also concurs with the findings in Chapter three of this work, where *T. vivax* was the only trypanosome species recorded in the migratory herd studied. Previous studies on the Jos Plateau e.g. Dede *et al.* (2005) and Kalu (1991) had found much lower prevalence of trypanosomes in tsetse by dissection and microscopy, but even with this lower prevalence, *T. vivax* was still the dominant species detected via microscopy. The findings of the current investigation contrast with Majekodunmi (2006), who reported a prevalence of 40.7% in tsetse using PCR, with no such overwhelming preponderance of any one particular trypanosome species. While the individual species make up of infections does differ, it is important to note this work was conducted several years prior to the current work, and the overall prevalence of 40.7% is not very different from the result obtained in the present study.

The observation has been made that *G. palpalis* is a particularly poor vector of *T. congolense* and previous experiments have demonstrated that it is difficult and sometimes impossible to infect species of the *palpalis* group with trypanosomes of the *congolense* group (Godfrey, 1966; Harley and Wilson, 1968). Thus, it is not surprising that *T. congolense* DNA was detected in only one of the 149 flies examined from the Jos Plateau. At first glance, the results from Yankari seem to contradict this slightly, since *T. congolense* was detected in *G. palpalis* from this area. However there are several observations that help explain this apparent contradiction;

1. The diversity of wildlife species in Yankari for tsetse to feed on is much greater than on the Jos Plateau, which translates into greater diversity of trypanosome species infecting tsetse. These findings are supported by other studies where wildlife are the main blood meal source for tsetse (Auty *et al.*, 2012, Anderson *et al.*, 2012).
2. The prevalence of *T. congolense* in *G. p. palpalis* is lower than in *G. tachinoides* and in *G. m. submorsitans*, supporting the supposition it is not an efficient vector for this species of trypanosome, see figure 4.2. *T. congolense* was detected at similar low levels in *G. p. palpalis* from Yankari Game Reserve using ITS PCR (Clement *et al.*, 2013).
3. Although *T. congolense* was detected in *G. palpalis*, it was predominantly present in mixed infections (60% of all *T. congolense* infections in this species of fly were mixed infections), adding further evidence to suggest limited vectorial capacity of *G. p. palpalis* for *T. congolense* alone.

#### 4.7.1.2 Infection rate by village and sex

The trypanosome prevalence was significantly higher in flies from Assop compared to those from Mangar and from Gyel Bali. This might be due to the smaller number of flies trapped and examined from Mangar or it might be related to differences in the prevalence of trypanosomes in the cattle host populations in these villages. It is also important to note that the number of flies trapped at each village location was variable; the highest number of flies and therefore the narrower confidence intervals are in the three villages where significant differences were detected. The other four of the seven villages had tsetse catch number less than 10, and so the significance (or lack of significance) in differences in the trypanosome prevalence in flies from those locations must be interpreted with caution.

The differences between males and females in terms of trypanosome infections are most marked with *Trypanozoon* infections; with male flies more likely to mature an infection in the salivary glands when compared to female flies (Maudlin *et al.* 1990). In this study, the overall trend in the villages of the Jos Plateau was a slightly higher (but insignificant) prevalence in females compared to males. Given that there were no *Trypanozoon* infections detected on the plateau and very few detected in flies from the Yankari Game Reserve it would have been difficult to detect a difference. It is also important to note that the protocol used in the current work would not have enabled detection of mature *Trypanozoon* infections as the salivary glands would have been present in the abdomen section which was part of the same material as was extracted for the midgut. In terms of *T. vivax* there is no difference between mature infection rates between male and female flies (Maudlin *et al.* 1990).

#### **4.7.1.3 Potentially transmissible infections**

As earlier noted, detection of trypanosomes in tsetse by PCR does not allow the determination of infection status with regard to whether an infection is active or not. However, by separating the mouthparts from the gut during DNA extraction and PCR, it was possible to determine whether infections occurred either in mouthparts, in gut or in both; allowing some inference to be made about whether the detected infections were mature or had the potential to develop into mature infections. It is also possible to identify trypanosome sub-species and detect mixed infections of two or more species (Masiga *et al.*, 1992; Morlais *et al.*, 1998).

In the present study, 44 potentially transmissible *T. vivax* infections were detected in Jos Plateau tsetse. Similarly, in Yankari, the 5 potentially transmissible *T. vivax* infections was less than the 11 overall detected for *G. palpalis*. If this study had been done using dissection and microscopy, these infections may have been wrongly identified on the basis of location alone. Knowledge of the likely transmissibility of these infections detected by PCR might be useful in future to model AAT risk in terms of the proportion of infected flies which could be expected to harbour mature *T. vivax* infections, and to better inform control strategies.

#### **4.7.1.4 Mixed trypanosome infections**

*T. vivax* was present in almost all trypanosome DNA positive infections in the Jos Plateau, excluding the possibility of mixed infections entirely. However in Yankari Game Reserve a number of different trypanosome species were detected, and mixed infections involving more than one species of trypanosome within a single tsetse fly

were detected. This finding is not unexpected given the availability of multiple wildlife hosts, which are often reservoirs of more trypanosome species diversity than domestic hosts (Adams *et al.*, 2006, Jamonneau *et al.*, 2004). This finding also highlights the potential risk posed to cattle migrating in the vicinity of the Yankari Game Reserve.

#### **4.7.2 Prevalence of *Sodalis glossinidius***

The prevalence of *S. glossinidius* was significantly higher in flies from the Jos Plateau than in those from Yankari Game Reserve. Even when the comparison is made between *G. p. palpalis* in both sites, this difference is maintained. It is difficult to propose a reason for this wide difference but it suggests that the two populations are quite isolated from one another with little gene flow between them. Within the Jos Plateau as well, there was a significant difference in the prevalence of *S. glossinidius* between Assop, Mangar and Gyel Bali which then shows that even across relatively small geographical distances, the infection dynamics between tsetse and *S. glossinidius* are highly variable.

In the study by Farikou *et al.* (2010), a significant association was found between the presence of this symbiont and trypanosome infections although Dennis *et al.* (2014) did not find the same positive association in their study. It was impossible in the current study to examine if such a relationship existed as *T. vivax* which only develops in the mouthparts of tsetse was nearly the only trypanosome species present in the Jos Plateau and no mid-gut infections of either *brucei* or *congolense* type

infections were detected. In flies from Yankari Game Reserve, the extremely low prevalence of *S. glossinidius* inhibited further statistical analysis to determine positive or negative associations between endosymbiont and trypanosome prevalence. Perhaps the presence of such an association can be explored in future studies involving more flies not only from the Jos Plateau but from other parts of Nigeria as well. It would also be useful to look for genetic differences in the *S. glossinidius* strains which might be present in the different species of tsetse from different regions of the country as it has been demonstrated by Geiger *et al.* (2007) that such genetic differences rather than the mere presence of this symbiont might account for its effect on tsetse-trypanosome interactions.

#### **4.7.3 Absence of *Wolbachia***

*Wolbachia* is yet to be reported in *G. p. palpalis* but was recently reported in the closely related *G. p. gambiensis* from Mali and Senegal as well as several *morsitans* and *fuscus* group tsetse (Doudoumis, *et al.*, 2012). There has been only one study previously investigating *G. m. submorsitans* in terms of *Wolbachia* and no infections were found (O'Neill *et al.*, 1993). Thus, the possibility that it might be present in the present sample of *G. palpalis*, *G. tachinoides* and *G. m. submorsitans* was not ruled out and they were screened for this symbiont. However, despite employing the use of two separate PCR protocols with two different primer sets and targets, none of the flies from either Yankari or the Jos Plateau tested positive for *Wolbachia*.

It is still not clear why *Wolbachia* is rarely found in tsetse of the *palpalis* group although it has been suggested that it might be present at low densities which are not detectable using the current primers and PCRs available (Doudoumis *et al.* 2012). There may be other biological reasons why it has so far not managed to invade this group of tsetse as much as it has other tsetse groups which might be uncovered by further phylogenetic studies.



## **5 Biting flies and possible mechanical transmission of AAT on the Jos Plateau**

In order to elucidate the role of biting flies on the Plateau in trypanosome transmission it is important to establish if there are able to act as vectors. Similar studies to those undertaken by Desquesnes and Dia (2004) could be undertaken. In these studies, non-infected cattle were kept with infected cattle in fly proof areas and wild caught *Atylotus fuscipes* were introduced. Using this or a similar protocol, it might be possible to gauge the role that biting flies on the Plateau play in trypanosome transmission. It might also be useful to investigate which biting insects commonly land on the cattle kept on the Plateau and examine how many of the insects feed on the cattle. This might also help to identify whether a large number of tabanids attempt to feed. Using traps specifically designed to catch tabanids would also provide more reliable estimates as the biconical traps used in the current work are more effective for trapping tsetse flies- especially, riverine tsetse species.

## **6 General Discussion**

## 6.1 Introduction

The background to this thesis was the 2008 study by Majekodunmi *et al.* (2013) indicating that the prevalence of bovine trypanosomiasis on the Jos Plateau was widespread and at an all-time high of 46.8%. The high disease prevalence was demonstrated to have severe implications for the livelihoods of pastoralists in the area (Majekodunmi *et al.*, 2013b).

The main objective of the present study was to identify factors contributing to the transmission and spread of the disease among extensively grazed cattle on the Jos Plateau by focusing on the vectors involved and possible routes or cycles of transmission. Three major themes were examined namely tsetse distribution on the plateau, seasonal migration of cattle and the potential role of mechanical vectors. This is the first study to investigate these three themes in parallel to further the understanding of bovine trypanosomiasis epidemiology on the Jos Plateau.

The tsetse surveys described in chapter two, showed that tsetse were present at very low densities of less than one fly per trap per day in only three of 20 villages. This is contradictory to the idea that tsetse populations may be expanding on the plateau (Dede *et al.*, 2005; Majekodunmi *et al.*, 2013a). The findings of this study suggest that tsetse populations on the Jos Plateau are restricted and may in fact, be in decline. This is further supported by the absence of tsetse flies in some villages where they had been reported in the past.

Reduction in tsetse presence and density appear to be related to the destruction of riverine vegetation. In the surveyed villages much of the riverine vegetation, which constitutes the main habitat of *G. p. palpalis* and *G. tachinoides*, had undergone various degrees of fragmentation due to the establishment of farms along stream and river banks. In addition, pesticides are used frequently to control crop pests on such farms and these practices are likely to be among the factors responsible for the absence of tsetse in the majority of surveyed villages and the low apparent density of the flies recorded in those villages where tsetse flies were detected.

While it is acknowledged that not trapping any tsetse flies after 72 hours of sampling in an area does not prove the absence of tsetse (Leak, 1999), it does suggest quite strongly that tsetse flies were not present or present at extremely low densities. With such low tsetse densities, trypanosomiasis risk in such villages and on the plateau in general would be expected to be very low. Consequently, it would be expected that prevalence of AAT should be low and that the disease would not have such a wide distribution as observed in the 2008 survey reported by Majekodunmi *et al.* (2013a). However, the molecular analysis of both tsetse and biting flies captured on the Plateau showed *T. vivax* DNA was present in the bloodmeals suggesting the presence of this trypanosome species on the plateau.

Seasonal migration may account for the observed high prevalence and wide spatial spread of bovine AAT on the plateau and paradoxical low tsetse population. The line of enquiry was therefore redirected to explore the role of migration with regards to

increased risk of trypanosomiasis transmission, as the evidence suggested that transmission of trypanosomiasis by tsetse was limited on the Jos Plateau itself.

To examine the role of seasonal migration in the epidemiology of AAT on the Jos Plateau, one migratory herd from a village where no tsetse flies had been detected after 18 months of sampling, was studied longitudinally between June 2013 and March 2014. Tsetse surveys conducted along the migration route of this herd and at its final destination, which lies at an altitude of about 450 m at the foot of the southern escarpment of the plateau, revealed the presence of *G. p. palpalis*. And although the apparent density of the fly was low, over the 10 month period, more than half of the sampled animals in the herd were found to be infected with *T. vivax*. Most of the infections occurred during the dry season from the end of October to early March. This was interpreted to be due to increased contact between the animals and tsetse at watering points, which are restricted during this season. During the dry season, the number of watering points reduces and tsetse flies as well as cattle congregate in smaller areas. This increases opportunities for transmission through co-existence of high cattle and fly density.

In the migrating cattle that were sampled as well most of the tsetse flies collected from the plateau, *T. vivax* was the only trypanosome species detected using a generic PCR capable of identifying 11 trypanosome species and sub-species. This suggests that transmission of *T. vivax* on the plateau could be due mainly to *G. p. palpalis*, which has been shown to be an efficient vector of West African strains of the

parasite (Moloo *et al.*, 1987). *T. vivax* is mainly a bovid parasite and since there are no wild Bovidae on the Jos Plateau this would indicate that the transmission cycle of AAT on the Plateau is strictly between tsetse and domestic cattle.

However, the migration of cattle beyond the plateau to areas such as the Yankari Game Reserve, where the diversity of trypanosome species was markedly different from flies trapped on the Jos Plateau, could account for the high prevalence found by 9Majekodunmi *et al.* (2013a), especially *T. congolense*. Blench (2004) suggested that in the aftermath of the conflicts of the Jos Plateau some Fulani travelled to Bauchi state and settled on the margins of these reserves. The higher diversity of trypanosomes from flies in the Yankari Game Reserve is explained by an abundance of wild animals which are known to be reservoirs of animal trypanosomes (Jordan, 1965). This would suggest that animals migrating to areas like the Yankari Game Reserve would experience higher trypanosomiasis challenge and could potentially bring back infections to the Plateau.

An unexpected finding from the longitudinal survey of the migratory herd was that of the 39 animals shown to have been infected only 6 were PCR positive more than once over the 10 month sampling period. There could be several explanations for this. Firstly, the owner of the herd may have treated animals that appeared sick thereby clearing the infection (although the owner confirmed that he did not apply trypanocidal treatment during the period of study).

A second explanation is the fluctuating parasitaemia related to the natural course of *T. vivax* infection, which goes through peaks and troughs due to the balance between the host immune response and the trypanosomes VSG class switching (Nantulya, 1990). Hence there may have been times when the parasitaemia was suppressed to a threshold below the detection limit of the PCR technique used.

A further explanation is that even though the analytical sensitivity of the ITS PCR technique has been shown to be high (detection limit of 1 trypanosome per ml of blood), use of FTA cards may result in lower diagnostic sensitivity (Cox *et al.*, 2010). Indeed the inability to detect infections in the majority of cattle which were earlier shown to have been infected could be a shortcoming of the method used to store the blood samples (FTA cards) as it was previously shown that trypanosomes may not be evenly distributed across the matrix of the FTA cards used for blood sample storage (Ahmed *et al.*, 2013).

Animals in the chronic phase of infection have a low parasitaemia which may have been undetectable by the PCR technique used in this study. The rationale for this is that the blood volume spotted onto FTA cards is approximately 200 µl, from which five punctures are taken, hence the actual volume of blood analysed by PCR is very low, reducing the probability of detecting low levels of parasitaemia.

Despite its lower diagnostic sensitivity, the FTA technique has been demonstrated to be robust (Ahmed *et al.*, 2013) and the alternative (working from a 1 ml of blood

sample per cattle) was not logistically possible in this study due to cost limitations and issues of sample storage and importation of Nigerian samples to the UK for PCR analysis. The advantage of FTA cards is the fact that DNA is denatured which enables shipment of samples from Africa without the additional cost and delay of obtaining import permits. One point of note is that the current work used generic PCR, and that use of the more sensitive species specific PCR may have improved ability to detect infections during low parasitaemia.

As discussed above, migration likely plays a major role in the epidemiology of trypanosomiasis on the Jos Plateau, in that animals are infected during dry season migration to areas with higher densities of tsetse and other reservoir hosts such as wild animals. However, within the Plateau itself, especially in the villages where tsetse were not found, there is good reason to hypothesise that mechanical transmission by other vectors may be implicated. From the results presented in Chapter 5, it is clear that other biting flies occur on the Plateau at relatively high densities during the peak of the rainy season. *Stomoxys* was the most abundant and tabanidae, although detected, were sparse. These species are known to be capable of mechanically transmitting trypanosomes (Mihok *et al.*, 1995; Desquesnes and Dia, 2003a, 2003b). The detection of trypanosome DNA on the mouthparts of biting flies trapped in villages where tsetse flies were absent strongly suggests that they are implicated in transmission, although degree of involvement could not be ascertained. Further observational studies looking at density of these flies on animals as well as



the rate of interrupted meals (see Chapter 5 for relevance) might give a clearer indication of the significance of mechanical transmission.

## **6.2 Future perspectives and options for control**

Even though infection pressure on the Jos Plateau may reduce over time due to a declining tsetse population, the increase in frequency and duration of migration to areas of high tsetse density may sustain trypanosomiasis at a relatively high level. It could also lead to the introduction of more pathogenic strains of trypanosome particularly if cattle encroach on areas like the Yankari Game Reserve where there is an abundance of tsetse and wildlife reservoirs of trypanosomes.

Measures will therefore need to be taken to protect animals during migration, especially as lack of access to water and grazing on the Plateau means that migration is inevitable.

There are two approaches that could be employed for trypanosomiasis control on the Jos Plateau. Based on the findings of the longitudinal migratory study, transmission risk was highest during the dry season and this is attributed to the co-concentration of cattle and tsetse at watering points. Therefore applying insecticide to cattle during this period could lead to reductions in tsetse density in the migration areas. However, success of this approach would depend also on the density of cattle in the migration areas. It may be important therefore to determine the density of cattle at the various

destinations where pastoralists spend the dry season, as areas with low cattle density would not have much of an effect on tsetse despite application of insecticide.

Another important approach would be the use of trypanocides in manner similar to that used by the herd owner in the migration study, who gave cattle trypanosome prophylaxis prior to migration. At least for the rainy season, this herd had no trypanosomiasis infection, as between June and September, only one infection was detected. If animals are going to areas where it is known there is a higher tsetse challenge, treating the herd with a prophylactic trypanocide could offer protection against infection during the rainy season. When animals return from migration at the end of the dry season, the herd could then be treated with a curative trypanocide to clear any infections that may have been picked up during migration. It would be important to educate farmers on the appropriate use of these drugs to prevent the emergence of drug resistance (Sow *et al.*, 2012)

The owner of the herd that was studied appeared quite knowledgeable about trypanosomiasis and as was suggested in Chapter 3, he could facilitate the transfer of knowledge to other pastoralists as part of future control programs.

### **6.3 Recommendations for further work**

To fully understand the epidemiology of trypanosomiasis on the Jos Plateau, future studies need to focus on where cattle are migrating to and determine the risk of trypanosomiasis in these areas by conducting intensive tsetse surveys.

It would also be interesting to look at whether cattle are acquiring immunity to the strains of trypanosomes circulating on the Jos Plateau. Characterisation of the local strains and studies assessing virulence by measuring morbidity of cattle experimentally infected may provide some indication as to the capacity of cattle to control parasitaemia through acquired immunity to local strains.

Further work could look at the tsetse endosymbionts using more recently developed molecular techniques (Schneider *et al.* 2012) to verify the findings of Chapter 4 and shed more light on the current situation especially with regard to *Wolbachia*

## **7    References**

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